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THE ANNALS OF APPLIED BIOLOGY

THE OFFICIAL ORGAN OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

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AND

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WITH THE ASSISTANCE OF THE COUNCIL

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VOLUME VIII JUNE, 1921 No. 1

THE PROTECTION OF MEAT COMMODITIES AGAINST BLOWFLIES

BY R. A. WARDLE.

(*University of Manchester.*)

A SURVEY of the literature concerning the economic aspect of the Muscid genera comprised in the popular term "blowfly" will indicate that, although much work has been carried out with respect to the prevention of oviposition upon living animals, and although several recent papers have dealt very fully with the problem of trapping or poisoning Muscid flies in general, yet the question of directly preventing oviposition upon foodstuffs has been neglected somewhat and its importance overlooked.

In Manchester, the alternate periods of fine rain and sunshine that constitute a normal summer are especially favourable to blowflies. Swarms of such flies are particularly noticeable in those shops—butchers, fishmongers, fruiterers and the market stalls—where, from motives of ventilation, the window space is not enclosed by glass. In such shops the usual methods of fly-prevention are impracticable. Poison bait traps do not enhance the appearance of a window containing food commodities. Screens of mosquito netting impede the circulation of air, so necessary in this type of shop, and becoming quickly soiled by juices of meat or fruit, obscure the view of the purchaser.

Attempts at fly-exclusion are therefore almost non-existent. The butcher will assert that his meat rarely becomes flyblown, a statement based generally upon the popular belief that only tainted meat is attacked by blowflies, but supported somewhat as a fact by the custom of keeping meat overnight in a refrigerator and placing it upon the boards of the open window in a slightly chilled and coagulated condition. The fishmonger is protected to some extent by the use of ice slabs and the hose-pipe. The fruiterer is frankly indifferent.

CONDITIONS CONDUCIVE TO OVIPOSITION.

The factors which affect oviposition fall readily into two categories: (*a*) nature of the foodstuffs attacked, (*b*) meteorological conditions.

The range of substances attractive to blowflies is wide, comprising

particularly flesh foods, ripe or over-ripe fruits, sugars (fructose, honey, molasses), faeces, stale urine, etc., but the range of substances upon which such flies will lay eggs is limited. My own experiments, confined solely to substances exposed for sale as food in open shops, and in a condition fit for human consumption, indicated the following order of preference: raw liver, raw sheep kidney, raw lean mutton, raw lean beef, beef cooked but underdone, bacon (mild cured).

On the other hand, food commodities apparently inconducive to oviposition comprised: chilled meat, well-cooked meat, over-ripe fruit, preserved meats (sausages, tinned meats, salt bacon), tripe as prepared for retail sale, fresh fish, animal fats, cheese, shellfish. Fish was only attacked when far too stale for human food.

That is to say, the substances upon which the blowfly prefers to oviposit are those which contain animal proteins, in particular those proteins known as *albumins* and *globulins*.

Albuminoid substances are not attractive, attempts to induce oviposition upon such substances as gelatin, ox-eye, fish-skin, rabbit-skin, etc., being unsuccessful.

It is not sufficient that the foodstuff be moist. It would seem necessary that the protein content has not been coagulated by heat nor washed out by water or salt solution. Slices of raw liver, for example, are highly attractive to blowflies; if the liver, however, be well washed in water or soaked in weak brine, so as to clear away temporarily the exuding blood and muscle plasma, the power of attraction is lessened; if the slice be singed superficially with a Bunsen flame or allowed to desiccate naturally in the sunshine, the power of attraction goes, however moist the surface be kept afterwards. Freshly-cooked meat, however moist, does not readily attract blowflies. Again, tripe is always extremely moist when fresh, but the windows of shops containing this commodity are conspicuously free from blowflies; repeated attempts to induce *Calliphora* to oviposit upon tripe were unsuccessful, nor could larvae be prevailed upon to feed on this substance.

The stimulus to oviposition, whether olfactory or gustatory, would seem to exist in the exuding juices, blood and muscle plasma, of the substance attacked, and particularly does the source of attraction appear to be the muscle plasma, for blood, whether fresh or putrid, seems quite unattractive, an observation previously noted by Miss Lodge(4); there is no doubt, also, that blowflies are more strongly attracted by putrefying substances than by fresh, and Miss Lodge recommends as the most attractive trap-baits: liver + maggots, brain + maggots, fish + maggots,

hard-boiled egg + maggots, that is to say, animal proteins in the early stages of putrefaction brought about by the digestive juices of the maggots.

If the digestive ferment of the larva be an enzyme analogous to pepsin, that is to say be proteoclastic and not lipoclastic, a conclusion warranted by its inability to hydrolyse fats, then the only products from the hydrolysis of muscle plasma would be Secondary Protein Derivatives, particularly proteoses and peptones. Anaerobic bacterial action commences early, however, and even in freshly-killed meat the breaking down of the peptones into amino-acids and into later putrefaction bases is slowly taking place. In fresh muscle attacked by maggots, the proteoclastic enzyme *protase*, occurring naturally in muscle, is accelerated by the enzyme of the maggot, and the hydrolysis of the proteins to amino-acids and the decomposition of these into putrefaction bases are comparatively rapid, so that in the broth produced by the action of blowfly larvae upon meat, such bases as *indole*, *skatole*, *methylamine*, *putrescine*, *methylguanidine*, would occur early.

That blowflies are chemotropic to such bases seems indisputable when the attraction of such substances as faeces, stale urine or putrid yeast is considered; yet Miss Lodge found hard-boiled egg plus methyl indole, or fresh meat plus skatole, to be quite unattractive, and no special attraction was evinced for *trimethylamine*, *tyrosine*, *guanine*, and *dimethylamine*.

It would seem doubtful at any rate whether the power of attraction of such bases is sufficient to induce the fly to oviposit. My own attempts to bring about oviposition upon human faeces and putrid yeast were unsuccessful. Howlett, however, has induced a species of *Sarcophaga* to oviposit upon skatole, and Mellor(5) states that "*C. erythrocephala* laid eggs upon the baits—human faeces, plums, milk and sheep's noses—which were placed together in the same receptacle."

It must be noted too that blowflies will readily oviposit upon freshly-dressed meat where the amount of such putrefaction bases must be very slight.

Further experiments may indicate that the stimulus to oviposition must be looked for among the amino-acids, and that in *Calliphora* the stimulus may be chiefly gustatory, and in *Sarcophaga* olfactory, which would account for the difference in response to skatole.

Meteorological factors, if not of such direct importance as the food factors, nevertheless play an important part. According to Miss Lodge an attractive bait placed in the shade attracted no blowflies, although

they had been swarming round it when it was placed in the sun. In my own experiments, the baits were duplicated, one series being placed in the sunniest part of a large garden, another series being placed in the shadiest portion of an adjoining insectary, which, having side walls of large mesh chicken wire, was quite open to flies. Both batches were equally readily infested within a few minutes of exposing, but in few cases did oviposition occur upon the baits exposed to the sunshine, and even then the eggs were few and scattered, whereas the baits in the shade were readily blown.

This apparent discrepancy in observations may be explained by an undoubted difference in response to light between the *Calliphora* type of blowfly and the *Lucilia* type. The flies used by Miss Lodge were chiefly *Lucilia*. Among the flies that visited my baits, *Lucilia* was in the minority. In fact numerous samples taken from various parts of the city all showed a marked predominance of *Calliphora vomitoria*, this fly constituting on the average 75 per cent. of the sample, the remaining percentage being made up almost equally of *Calliphora erythrocephala* and *Lucilia caesar*. In addition to being in the minority, *Lucilia* had also to compete for foothold on the baits not only with *Calliphora* but with hordes of Acalyptrate Muscidae as well.

The difference in response to light has been pointed out by Herms (2): "The more frequent presence of *Calliphora vomitoria* in houses and like situations is due chiefly to a relatively low degree of responsiveness to light, so that odours from darker places may attract it more readily. On the other hand, *Lucilia caesar* is very strongly phototactic and consequently would seek the open, and if by chance it should find its way into darker places, its responsiveness to large luminous areas would soon lead it to escape. It may be assumed that the two species are equally chemotactic, which assumption is justified at least by observation. Since *C. vomitoria* is less strongly phototactic, individuals least so might easily be attracted into fairly dark places and would not soon be compelled to leave, because of their phototropism."

This difference in habit was strikingly observed during the hot August sunshine of 1916 in the trenches outside Delville Wood, on the Somme, where blowflies were abnormally abundant. In the shade afforded by the deeper portion of the trench, round the traverses, any moist patch on the chalk wall would be hidden by a dense indigo-coloured cluster of *Calliphora*, large as a soup plate, unredeemed by the metallic green of *Lucilia*, whereas, where the trench was shallow or blown in, the green shimmer of *Lucilia* was everywhere.

It would appear too that *Lucilia* prefers carrion in bulk and is primarily a fly infesting carcases in the open, thus not having the economic significance of *Calliphora* as regards foodstuffs.

The hygrometric condition of the atmosphere is not without significance. In continuously fine dry weather, oviposition occurred more frequently in the early morning between dawn and 8 a.m., whilst the atmosphere was somewhat moist. A sunny morning following a wet night, or a sunny afternoon following a wet morning, were particularly favourable.

Wind was antagonistic. Few flies were on the wing and the baits became dry and unattractive.

Experiments in prevention.

Screening and trapping, as already pointed out, are not methods readily applicable to the problem in hand. Attention was therefore directed to the question of repellent substances.

These fall into two classes:

(1) Substances directly applicable to foodstuffs.

(2) Substances indirectly applicable to foodstuffs.

The range of repellent substances that can be directly applied is limited. A trial was made with the household remedies usually recommended against flyblow, such as vinegar, onion juice, pepper, salt, tomato juice, etc., and the list was extended by the addition of formalin, boracic acid, and a proprietary article termed Milton, having a slight chlorine odour. Slices of raw kidney or liver were sprayed with or dipped in solutions of these substances and exposed, one batch in the sunshine, one batch in the shade. The results obtained from the baits exposed in the sunshine were of little use as even the controls were rarely blown. Flies fed freely on all the samples but particularly on those treated with vinegar, weak Milton, and borax. The samples treated with concentrated Milton and with $2\frac{1}{2}$ per cent. formalin were avoided when flies were few, when the number of flies increased these samples were attacked also.

The following table is typical of the oviposition results observed in twenty repetitions of the experiment.

The weak solution of Milton would appear to be more repellent than the strong but other experiments did not confirm this. In fact, weak Milton and vinegar seemed slightly attractive. The success of pepper appears due to the drying effect produced upon the surface of the bait. It was not so effective when applied to liver or to very juicy meat. Precipitated chalk gave similar results. Cooper and Walling(3), experi-

menting with a wide range of repellent substances against blowflies, used precipitated chalk as a vehicle for the chemicals. In most cases, the chalk plus chemical conferred immunity during one to four days, complete immunity being obtained by the use of copper carbonate, nitrobenzene, picric acid, creosote, sinapis oil and aniseed oil. It must be noted, however, that precipitated chalk by itself will protect fresh meat so long as it remains unsaturated by exuding juices either of muscle plasma or of putrefaction, and that when chemicals are present that can exert an inhibiting action upon bacterial activity, putrefaction is delayed and the chalk will last a long time before becoming saturated. That is to say, the substances recommended by Cooper and Walling may not necessarily be repellent to the fly, the apparent repellent action being due possibly to the restraining effect exerted by the chemicals upon bacterial activity, and the action of the precipitated chalk in keeping the meat surface dry.

Sliced sheep's kidney placed in insectary at noon	Weather sunny, cool in afternoon. Wet night, fine morning.	
	Dusk	9 a.m. next day
Untreated...	+slightly	+heavily
5 % Milton	–	+slightly
Concentrated Milton	+four scattered eggs	+considerably
Concentrated vinegar	–	+considerably
Formalin 2½ %	–	+slightly
Pepper, dry	–	–
Boracic acid, dry...	+slightly	+considerably
Boracic acid solution, strong ...	+considerably	+heavily
Onion juice	–	+heavily
Lemon juice	+slightly	+heavily
Salt, dry	–	+slightly
Salt, solution	–	+considerably
Formic acid, 5 %	–	+slightly

+signifies blown. –signifies not blown.
"Heavily" signifies three or more clusters of eggs.
"Considerably" signifies one or two clusters of eggs.
"Slightly" signifies scattered eggs, rarely exceeding 12 or 15.

In the case of powdered boracic acid or powdered salt, the drying effect is soon lost owing to the solvent action of the meat juices and no success, as repellents, was observed in my experiments with these substances.

Cooper and Walling, and Miss Lodge, however, assert positively that boracic acid is repellent to blowflies.

Weak formalin was decidedly repellent, but hardly applicable to meat commodities.

The second class of repellent substances, namely such as are indirectly applicable to meat commodities, comprises such substances as have a distinct and penetrating odour, the essential oils, phenols, paraffins, esters, etc. For example, the following list of substances was found by Miss Lodge to be decidedly repellent: *pipendine*, *oenanthol*, *xylol*, *amyl acetate*, *methyl salicylate*, *anisole*, *citral* (strong), *ethyl sulphocyanide*, *oil of thyme*, *of cassia*, *of Java citronella*, *of palma rosa*, *of bay*, *of heliotrope*, *of lavender*, *of cinnamon leaf*, *of cinnamon bark*, *of sassafras*, *of cloves*, *of camphor*.

Cooper and Walling give: *methyl salicylate*, *p-nitraniline*, *picric acid*, *creosote*, *green oil*, *boracic acid*, *mustard oil*, *sod oil*, *iodoform*, *di-methylaniline*, *quinoline*, *allyl alcohol*, *fusel oil*, *pine oil*, *alizarine oil*, *origanum oil*, *sinapis oil*, *alloin*, *saponin*, *copper carbonate*, *nitrobenzene*, *aniseed oil*.

Now a substance may be repellent to a fly attracted to the bait from motives of curiosity or hunger, without being necessarily repellent to a female fly under the far stronger oviposition-impulse. Many of the substances listed above are, further, obviously unsuitable for use in proximity to foodstuffs, and others are too expensive or difficult to procure for the average food vendor.

A selection of substances was made therefore, and, as in practice it would be undesirable to apply them directly to foodstuffs, an indirect method of testing them was adopted. The food samples were placed within cylindrical glass dishes over the opening of which a piece of cotton twine netting was tied, after being smeared with the substance to be tested. The netting was of quarter-inch diamond mesh, wide enough to allow the largest *Calliphora* to slip in and out of the dish; as the dishes were of glass, equal illumination of both sides of the netting was obtained and the possibility of the mesh acting as a mechanical obstacle to the ingress of the flies thus excluded. It has been asserted by Spence (1) that flies can be prevented from entering a room through open doors and windows by covering these openings with wide-mesh netting, even herring netting, provided there is no source of illumination behind the door or window. In my later experiments, however, a box of three feet cubic dimensions, lined with black cloth and completely closed except for a narrow slit, was readily entered by large numbers of *Calliphora*, and was also entered quite easily when one side was removed and replaced by black netting.

To perfume the netting, it was found quite sufficient to moisten the palms of the hands and to roll the netting between them. Substances selected were the more easily obtainable essential oils—cloves, citronella,

cinnamon, aniseed, etc. In addition, formic acid, picric acid, boracic acid, and nitrobenzene were tried. Phenolic and paraffin compounds were not tried as their use in proximity to foodstuffs is scarcely practicable.

The following table is typical of some twenty repetitions of the experiments.

Sliced ox liver placed in insectary at noon	Hot afternoon, rain during night, fine hot day succeeding	
	Dusk	Noon next day
Untreated ...	+ heavily	+ heavily
Oil of cloves ...	+ considerably	+ considerably
„ aniseed ...	–	–
„ eucalyptus	–	+ considerably
„ almonds...	+ heavily	+ heavily
„ citronella	+ considerably	+ considerably
„ cinnamon	+ considerably	+ considerably
Formic acid ...	–	+ considerably
Boracic acid ...	+ considerably	+ considerably
Picric acid ...	+ heavily	+ heavily
Nitrobenzene ...	+ considerably	+ considerably

In most cases, the majority of the samples were blown within six hours, if weather conditions were suitable. Samples protected with eucalyptus oil, formic acid and sometimes clove oil, remained untouched for twelve hours, but were generally attacked after that interval.

Similar results were obtained when the baits were placed in a hothouse, average temperature 78° F., into which blowflies could readily enter.

The trials with aniseed oil were repeated on a larger scale. A miniature market stall was erected. It was divided into two halves by a partition. One half was enclosed on all sides, except the mesh side, by dark material, so as to form a semi-dark chamber; the other half was similarly enclosed by white mosquito netting so as to be quite exposed to sunlight. The cotton netting, after being smeared with oil, was hung loosely upon protruding tacks. A variety of baits was placed within the compartments. As before, the baits placed within the light compartment, though visited by clouds of blowflies, were rarely blown.

In no case were the baits that were protected with aniseed oil blown within twenty-four hours.

Various types of netting were tried but in no case was greater success obtained than with the quarter-inch mesh. The advantage of cotton netting, apart from the fact that it is cheaper than netting made from other material, costing from sixpence to eighteen-pence per square yard

according to thickness of thread, is that it absorbs the oil readily, and, owing to its diamond pattern, is fairly elastic. The oil is fairly expensive if purchased from the retail pharmacist, but can be obtained from W. J. Bush et Cie, Grasse, Alpes Maritimes, or from their Hackney branch, at 3*s.* 9*d.* per lb. or at a lower price for quantity. The exact specification is Oil Aniseed, China Star. Oil of sweet fennel would be probably just as effective in spite of its lower percentage of anethole, but costs 10*s.* per lb. A pound of the oil goes a long way, however, if applied in the manner described.

There would seem little advantage to be gained by diluting with methylated spirits or emulsifying with soap solution. Hasty and interrupted experiments seemed to indicate that the efficiency of emulsions of the oil varies directly in proportion to the percentage of oil present. Thus a 50 per cent. emulsion was not effective after twelve hours, a 25 per cent. emulsion not effective beyond six hours, and an emulsion containing less than 10 per cent. of oil not reliable at all. However, accurate work is needed to clear up this point.

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ON THE FUNGUS FLORA OF GLASSHOUSE WATER SUPPLIES IN RELATION TO PLANT DISEASE[1]

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(With 1 Text-figure.)

It has long been recognised that the elimination of infection centres is an important factor in the control of disease, but while the intimate relation between polluted water and epidemics of human diseases is now common knowledge, little attention has yet been given to the corresponding relation between water contaminations and plant diseases. Attention was drawn to this latter problem by a severe epidemic of "damping-off" in tomato seedlings in the early part of 1919 and preliminary experiments showed that the causal organism, *Phytophthora cryptogea* Pethybr. (5), was carried by the water used in cultivation. Later in the year the same fact was proved in the case of a severe attack of "buck-eye" rot of tomatoes caused by *Phytophthora parasitica* Dastur (3). It was evident that a centre of infection existed in the water supply and this appeared of sufficient importance to justify further study.

Sources of water.

The water necessary for glasshouse cultivation is pumped from a well or other source of supply into a galvanised iron or painted steel tank placed at from 25 to 30 feet above ground. Here it is stored for a varying period and thence conveyed direct to the house by means of piping and hoses. In some nurseries covered tanks are used, but this is by no means a constant practice. Uncovered tanks are open to contamination by air-borne bacteria and fungus spores, as well as by debris dropped by birds, which go there to drink. The sources from which the water is drawn in the Lea Valley are the Water Company's supply,

[1] A grant in aid of publication has been received for this communication.

deep bricked artesian wells, shallow surface wells, brooks and ponds. The position of the wells or other source of supply with regard to the actual houses varies considerably, some being placed in a central position surrounded by glasshouses while others are at various distances away. Analyses have been made of water from wells, etc., of different types and position in order to ascertain how far these factors affect its purity as regards contamination with pathogenic micro-organisms.

Experimental.

A reliable method of sampling was devised by filtering large volumes of water through a filter prepared in the following manner, and examining

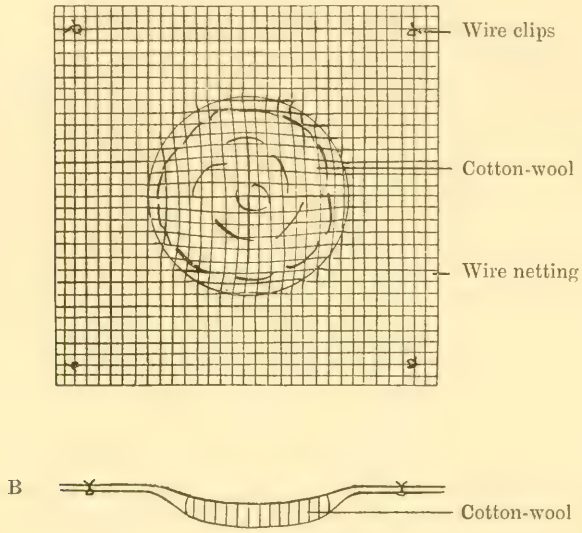

Fig. 1. Filter.
A. Plan. B. Section.

the debris collected. A piece of wire netting, eight inches square, with four meshes to the inch, was cupped in the centre, making a circular depression four inches in diameter and three quarters of an inch deep, Fig. 1. A layer of absorbent wool about a fifth of an inch thick was placed in the circular depression and held in place by another piece of netting similar to the first, but having the central depression only half an inch deep. The two pieces of netting were firmly fixed together at the four corners by means of copper wire, the filter wrapped in brown paper and sterilised. To obtain the necessary sample, the sterile filter was secured over the outlet hole of the tank when the latter was empty,

and the tank then filled and approximately 2,000 gallons of water allowed to pass through the filter. The outlets vary from four to eight inches in diameter and much of the water passed through the netting beyond the wool. This was necessary to prevent the filter from bending, but sufficient water passed through the cotton-wool to yield an adequate supply of bacteria and fungus spores. When the tank was empty, the filter was removed, wrapped in a new piece of sterile brown paper and taken to the laboratory for examination. In sampling the water direct from the main, from brooks and ponds, the filter was fixed under the inlet pipe which fills the tank, protection from aerial infection being provided by tying a piece of sterile paper above the pipe and filter. The cotton-wool plug and the debris held by it was transferred as quickly as possible to 500 c.c. of physiological salt solution, shaken for five minutes and dilutions of 1/25 and 1/250 then made in further salt solutions. 1 c.c. was removed from each dilution and plated upon the following media: (1) Quaker Oat Agar (Clinton)(2), (2) Modified Egg-Albumen Agar (Waksman)(6), (3) Cook's Agar No. II (Cook)(1), (4) Potato Agar.

Potato agar yields a rapid luxuriant growth suitable for preliminary examination, but the plates are often too badly contaminated with bacteria to allow the slow-growing fungi to develop. Quaker Oat agar is specially suited for determining the presence of the various species of *Phytophthora* but if any of the Mucorales are present they develop too rapidly and suppress the development of the more slowly-growing species. Cook's agar proved to be an excellent medium for the work. The various organisms, except the species of *Phytophthora*, develop rapidly and are readily distinguished; pycnidial forms are specially well shown. The Mucorales grow rapidly and if large numbers are present they tend to obscure the other fungi. Modified egg-albumen agar produces a slow growth of all the fungi. It is suitable for careful study and isolation of the various colonies. The plating of the water samples was carried out in the usual manner, special care being taken to cool the melted agars where possible to 45° C., because of the delicacy of some fungus spores. All plates were incubated at 22° C. and examined every 24 hours, only the actual parasites being identified. Doubtful organisms were isolated in pure culture and tested for pathogenicity by inoculation. By utilising all four media a reasonably accurate qualitative estimate of the fungus flora of the water sample was obtained.

In Table I representative water samples are given, and a careful examination appears to justify the conclusion that the water supply may be so seriously contaminated as to constitute an important source

Table I.

Source of water sample arranged in order of purity	Number of samples tested	No. of times each fungus found in total number of samples: Botrytis cinerea	Cladosporium cucumerinum	C. fulvum	C. herbarum	Fusarium sp.	Macrosporium solani	Phytophthora cryptogea	Ph. parasitica	Phoma sp.	Rhizoctonia solani	Rhizopus nigricans	Verticillium albo-atrum	Average number of fungi per sample	Remarks
Water Company, direct from main	4	.	.	.	1	.	.	.	.	4	.	.	.	1·25	These records may be actually contaminations in the water supply, but are more likely to be nozzle contaminations
Water Company, covered tank ...	4	1	.	.	1	.	.	.	.	4	.	.	.	1·50	As above
Deep artesian well	2	.	.	.	1	1	.	.	.	1	.	.	.	1·50	These contaminations are possibly from the air, during filtration
Deep well	6	.	.	1	6	4	4	.	.	3	3	2	1	4·00	Faulty brickwork allows surface contamination
Water Company, uncovered tank	6	4	.	3	6	1	2	.	.	6	.	4	2	4·70	
Brook after cleaning	1	1	.	.	1	1	.	.	.	.	.	1	1	5·00	Vegetation was removed and banks cut clean
Shallow well clear of houses ...	7	2	.	4	7	7	.	2	2	4	2	7	.	5·30	
Shallow well surrounded by houses	6	4	1	1	6	6	3	2	1	2	3	5	5	6·50	
Shallow well surrounded by houses; high organic content	2	2	.	.	2	2	1	1	1	2	1	1	2	7·50	The filter was covered with a dense black slimy deposit
Brook before cleaning	1	1	.	.	1	1	1	.	1	.	1	1	1	8·00	Brook was shallow and full of vegetation
Pond	2	2	.	.	2	2	2	1	1	2	1	2	1	8·00	Diameters 10 and 15 yards approx.
Relative frequency of pathogens in Lea Valley waters	41	17	1	9	34	25	13	6	6	28	11	23	13		

of infection. The enumeration of the various members constituting the microscopic population of the water samples are necessarily incomplete, for whilst it is possible readily to distinguish some members, others such as *Phytophthora cryptogea* or *Colletotrichum oligochaetum* are identifiable in mixed culture only with difficulty. Owing to the uncertainty in determining the latter it has been omitted from the table. The identification of *Bacillus lathyri* (4), causative of "stripe" of tomatoes, is a lengthy process, but in six samples this was attempted and the bacillus found in three. With present technique, quantitative results do not seem possible and consequently in comparing the fungal populations of the various samples, the number of different species of parasites found has been taken as an indication of the purity of the water.

The results given in Table I clearly show that the extent of fungoid contamination of the waters varies with the source from which they are drawn. The purest waters are those from the Water Company (Metropolitan Water Board) and deep artesian wells; there being an average of 1·25 to 1·50 pathogens per sample. It is possible too that the fungi found in these waters may be nozzle contaminations rather than actual contaminations of the water supply. Water which had passed through an uncovered tank was more contaminated than that from a covered one. There is evidence that the former collects fungus spores and becomes a source of contamination to the water passing through. Samples from wells into which the surface drainage can readily pass yield a large number of species. Deep wells of this kind were only slightly less contaminated than shallow wells, but shallow wells placed some distance away from the glasshouses were generally purer than those surrounded by houses. Again, those wells, whose waters contained a large amount of decaying organic matter, were richer in fungi than those whose waters were clearer. Water from brooks and ponds was also highly contaminated, but a slight reduction in the number of fungus species resulted from thoroughly clearing away the vegetation. It will readily be seen that the Water Company's supply and water drawn from deep artesian wells are comparatively free from fungoid contaminations, while water from wells polluted by surface drainage, as well as brooks and ponds, may be a very serious source of infection. The various species of *Phytophthora* appeared in these latter sources.

Isolations.

Representative isolations of the various pathogens were made, grown in pure culture and tested for pathogenicity. To ascertain the

extent of the growth, which could be made by the various fungi in the actual well waters, samples of water were obtained from six different wells; the samples varying in the quantity of organic matter they contained. Tubes were filled at the rate of 10 c.c. per tube, plugged, sterilised and inoculated from the pure cultures of the fungi isolated from the water samples. In every case an appreciable growth resulted within 20 days and within 10 days the growth was sufficiently strong to show that the waters tested were moderately good media for the growth of the pathogens. There was a striking relation between the rate of growth and the amount of organic matter in the water; the rate of growth in the clearer waters being slower than that in the impure waters.

The purification of contaminated waters.

The purification of contaminated waters may be attempted along three main lines, namely filtration, sterilisation by heat or sterilisation by chemicals.

Filtration.

The beneficial results obtained by this method are patent when one considers the efficiency of the filter beds utilised in the preparation of pure water for domestic purposes. To be efficient, such filters require constant overhauling and must be of fairly large extent and thus the utilisation of filter beds on any but the largest nurseries does not come within the limits of practice. Certain means of roughly filtering contaminated waters have been attempted with beneficial results. In one case the contaminated water was allowed to percolate through a tightly packed sand and coke filter, some two feet in diameter and six feet in length. The resulting water proved to be free from fungus spores for the first three months during which the filter was in use. After 12 months' use the efficiency of the filter was slightly reduced, but there was still a great reduction in the number of fungi present in the water after filtration.

Sterilisation by chemicals.

In view of the desirability of obtaining results fairly rapidly, it was only possible to attempt a rough determination of the toxic action of various chemical compounds upon the vegetative and "summer" spore forms of fungi selected owing to their comparatively great commercial importance as disease producers. The type of fungus growth used in the tests was as follow:

Pieces of mycelium of *Phytophthora cryptogea*, in such quantities as are easily picked from a culture by an ordinary platinum needle, were

taken from luxuriantly growing cultures from three to eight weeks old, on Quaker Oat agar. With *Phytophthora parasitica*, small pieces of mycelium as before, but bearing numerous ripe sporangia, were placed in sterile water for an hour before the chemical was added, so that numerous zoospores were liberated. The strain of *Colletotrichum oligochaetum* used was isolated from lesions on cucumber leaves from a commercial nursery in 1920. On Quaker Oat agar acervuli are produced, yielding large quantities of spores, and a suspension of these was prepared by pouring 10 c.c. of sterile water into a tube containing a culture, gently shaking and pouring off into a sterile tube. For *Cladosporium fulvum* suspensions of spores were made in sterile water under conditions as aseptic as possible by scraping the spores from "mildew" spots on freshly gathered tomato leaves. Control plates gave comparatively pure growths of *Cladosporium fulvum*.

In carrying out the tests, the suspension of mycelium or spores was added in 1 c.c. lots to sterile test-tubes. The necessary amounts of sterile water and of solutions of the chemical were added to bring the final volume up to 2 c.c. at the desired strength. After shaking well the suspensions of spores and chemicals were allowed to stand for about 20 hours. Control tubes were set up in each case and invariably gave rise to good growths when plated. After standing for 20 hours the suspensions were transferred to sterile centrifuge tubes, diluted to 10 c.c. with sterile water, centrifuged, and after decanting the top 9 c.c. the remaining 1 c.c. containing mycelium or spores was plated—*Phytophthora* spp. on Quaker Oat agar and *Colletotrichum* and *Cladosporium* on Waksman's modified egg-albumen agar. The plates were incubated at temperatures approximating to the optimum for the particular fungus concerned, viz. *Phytophthora parasitica* at 29° C., *P. cryptogea* at 25° C., *Collectotrichum oligochaetum* at 25° C., and *Cladosporium fulvum* at 22° C.

Sterilisation by heat.

The suspension of mycelium or spores was placed in a sterile test-tube and heated for one minute in a water bath at different temperatures; the latter being recorded by means of a thermometer in a second test-tube in the water bath. After heating, the suspension was plated out and incubated as in the chemical tests.

The results in Table II show the limits within which the lethal concentration lies expressed as parts per 100,000 of chemical in the water suspension. The first number in the columns indicates the concentration at which the compound begins to have a toxic effect and the

Table II.

Table showing the limits within which the death-point lies expressed as parts per 100,000 *of chemical in the water suspension (all liquids were measured by volume and solids by weight).*

	Phytophthora parasitica Mycelium and conidia	Phytophthora cryptogea Mycelium	Colletotrichum oligochaetum Spores	Cladosporium fulvum Spores
Mercuric chloride	5–20	5–20	0·5–1	1–2
"Chloros*"	25–50	20–50	1–5	35–50
Formaldehyde	10–50	5–50	50–70	100–300
Formic acid	10–50	10–50	10–50	50–200
Potassium permanganate ...	40–100	40–100	5–350	10–500
Copper sulphate	10–50	10–40	10–40	40–100
Ammonium polysulphide solution, 1919	100–300	100–200	10–30	10–30
Phenol	300–500	300–500	70–200	70–200
Sulphuric acid	100–500	5–30	100–500	500–2000
Calcium bisulphite solution ...	300–1000	300–1000	100–300	100–300
Potassium sulphide	over 2000	1000–2000	10–50	50–200
Acetic acid	100–200	50–200	50–200	200–300
Ferric chloride	50–2000	50–200	600–2000	600–2000
Ferrous sulphate	over 2000	over 2000	over 2000	over 2000
Zinc chloride	„ 3000	„ 3000	„ 3000	„ 3000
Hexamine	„ 3000	„ 3000	„ 3000	„ 3000
Potassium sulphate	„ 3000	„ 3000	„ 3000	„ 3000
Potassium biphosphate ...	„ 5000	„ 5000	„ 5000	„ 5000
Sodium carbonate	„ 5000	1500–5000	„ 5000	„ 5000
Sodium formate	„ 5000	over 5000	„ 5000	„ 5000
Sodium nitrate	„ 10000	„ 10000	„ 10000	„ 10000
Temperature to which water must be raised to kill mycelium and "summer" spores	60° C.	50° C.	60° C.	70° C.

* Proprietary solution.

second number the concentration which is completely toxic. Thus five parts in 100,000 mercuric chloride retards the growth of *Phytophthora parasitica*, while 10 parts in 100,000 kills the spores and mycelium. Where "over 2000" appears in a column, it indicates that the number following "over" is the lowest concentration which shows any toxic effect. The final lethal concentration in these cases is too high for practical purposes and has not been determined. The compounds are arranged in order of relative toxicity; the most effective ones appearing at the top of the list. It must be understood that the concentrations given are toxic only to the vegetative and thin-walled spores of the fungi employed, and not to the thick-walled resting spores. Thus, while they may not completely rid the water of its infections, they practically do so by

killing the mycelium and "summer" spores which it contains. Only the thick-walled resting spores remain and these have not been found to any great extent in the water samples tested.

By raising the temperature of the water to 70° C., for one minute, the mycelial and "summer" spore forms of the fungi tested were killed. Complete sterilisation of the water is obtained by raising the temperature to boiling point for one minute.

Control.

Investigation of the cause and source of any plant disease is not complete unless the water supply has been carefully examined as a possible source of infection and in cases where it is probable that this is contaminated, steps must be taken to purify it.

The first process should be the thorough cleansing of the well, by removing as much algae and decaying plant material as possible, for a surface scum of the former assists the fungi mechanically and the latter makes the water a good medium for fungal growth. Copper sulphate used at the rate of 10 lb. to 1,000,000 gallons of water will keep down the growth of algae, which are so objectionable in wells, tanks, etc. When the water is very thick with particles of decaying plant material it may be cleared by precipitation with alum and sodium carbonate, added at the rate of 18 lb. potash alum and 5 lb. sodium carbonate for every 25,000 gallons of water. Perchloride of iron at the rate of 9 lb. per 25,000 gallons of water is also a useful clarifying agent. Of the chemical compounds tested, mercuric chloride and "chloros" are the most toxic to fungi. Mercuric chloride, however, is highly poisonous to man and it is not advisable to use it unless very special precautions are taken to prevent the workers drinking the treated water. No such restrictions apply to "chloros" in such a highly diluted condition. It should be added at the rate of 50 gallons to 100,000 of water, but half that concentration will give protection against the "summer" spore forms of *Phytophthora* spp. Complete sterilisation of the water is only obtained by boiling, but beneficial results have been obtained by arranging the pipes leading to the tank in such a manner that they are heated by passing through the boiler. While it may not be so necessary to supply the more mature plants with disease-free water, it is advisable to sterilise in some way all the water used in the early stages of propagation, when the plants so readily succumb to "damping-off" and "foot rot."

In choosing the source of water supply the following points should be considered. Stagnant brooks are really natural sewers and may be

badly contaminated with disease organisms. Ponds full of algae and weeds are suitable breeding places for fungi and bacteria. Shallow wells receive surface drainage from the neighbouring land and are liable to be contaminated, especially if heaps of decaying plants are deposited in their vicinity. Deep artesian wells are the purest and safest. Care should be taken to cover all wells and tanks to prevent contamination with debris that otherwise might gain entrance.

Summary.

1. Nursery waters in the Lea Valley have been examined and some shown to be an important source of disease.

2. Contaminated waters may be purified by filtering, boiling and by addition of certain chemicals.

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STUDIES IN BACTERIOSIS. V

FURTHER INVESTIGATION OF A SUGGESTED BACTERIOLYTIC ACTION IN *PROTEA CYNAROÏDES* AFFECTED WITH THE LEAF-SPOT DISEASE

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(With 1 Text-figure.)

In a previous paper[1] attention was drawn to the possible occurrence of a bacteriolytic process analogous to that found in animals. The existence of some such phenomenon was indicated by the marked localisation of the disease, by the difficulty experienced in the isolation of the parasite from the diseased areas, and by the appearance of the diseased tissue, the cells of which showed no well-defined micro-organisms. The presence of bacteria was suggested by the granular appearance of the contents of many of the cells, giving the impression of a bacterial zoogloea embedded in resinous material. The degree of granulation of this substance varied in different cells, and its affinity for bacterial stains varied in proportion to the degree of granulation. These observations seemed to suggest a process of dissolution of the bacterial zoogloea.

The present work was undertaken to elucidate this phenomenon and to determine the fate of the bacteria after they had invaded the leaf tissue and produced the *leaf-spot*.

Fresh isolations of the parasite, *Pseudomonas Proteamaculans* P. and S., were made and again the old difficulty was experienced; of twenty-two attempts, only five yielded cultures of the causal organism. It does not appear that any seasonal fluctuation could account for the numerous failures, since of the five successful attempts one was made in November, two in March, and two in July.

The method employed was as follows: The surface of the leaf was

[1] Paine, S. G. and Stansfield (1919). *Ann. App. Biol.* VI, p. 27.

washed rapidly with 65 per cent. alcohol, and a portion containing one of the diseased areas cut from the leaf and ground under aseptic conditions with sterile sand and 5 c.c. of sterile water. After a lapse of a minute or so for the settling of the sand, 1 c.c. of the liquid was further diluted with 4 c.c. of water, and 1 c.c. of this dilution was plated in bouillon agar. In the successful experiments the number of colonies of *P. Proteamaculans* was never great, and since these represented roughly one twenty-fifth of the total number in the leaf tissue taken, it must be concluded that the number of viable organisms present was much less than might have been expected considering the extent of the disorganisation of the tissue. The method may perhaps appear rather drastic and liable to loss of bacteria by adsorption on the sand and detritus and by their actual destruction in the grinding process. A certain amount of loss does occur, but as shown later (footnote, p. 23), it is not more than about 20 per cent. of the organisms present. It seems then that some factor militating against the bacteria comes into action after a lesion of a certain size has been produced.

In this case a large percentage of the spots might be expected to contain no viable organisms. To determine this percentage 100 "spots" were cut aseptically from leaves and dropped each into a separate tube containing 5 c.c. of bouillon; in order to aid the escape of bacteria each area of the diseased tissue was usually cut through the middle. After incubation for three days the broth in all cases remained perfectly clear and after streaking the broth from 75 tubes across the surface of nutrient agar plates, no growth of bacteria was obtained. The experiment was repeated with 64 diseased spots and again after three days none showed the presence of *P. Proteamaculans*, although 17 tubes showed turbidity by other organisms. Five of these examined after seven days' incubation still showed no development of *P. Proteamaculans*; two tubes, however, which had been put aside were found to contain this organism when examined ten weeks later. It seemed possible that the reddish-brown pigment which had diffused from the tissue and coloured the broth quite strongly in certain cases, or perhaps some other diffusible toxin from the leaf, had a retarding influence upon the rate of development of the organism. Six of the most strongly coloured tubes were therefore inoculated from a pure culture of *P. Proteamaculans*, and although growth was not very vigorous at first, all six developed the organism and were strongly turbid after a week or so. Marked retardation of growth was therefore not due to this cause. A third set of 65 tubes was prepared as before. At the end of nine days the broth in all cases was clear

and bright, but in 44 (and seven days later in 48) cases a colourless gelatinous substance was observed around the piece of tissue lying at the bottom of the tube. On staining, this appeared as a zoogloea of bacteria of similar shape to *P. Proteamaculans*, but smaller in size. This did not develop further and was interpreted as a swollen mass of the gum-like substance containing dead bacteria. This phenomenon of the swelling of the gum has been observed in the case of dead or dying leaves kept under moist conditions: small beads of the yellow gum were seen on the surface of the diseased spots, and these could be removed, spread on a slide and stained. They showed the same appearance of small embedded bacteria as described above. In order to test the viability of these organisms small masses of the gum of the size of a pin's head and containing some millions of the bacteria were ground with sterile sand and water, and the water then plated on agar and incubated. Four experiments of this kind were made; in three cases no growth was obtained, but in the fourth numerous colonies developed. The experiments differed in that the gum used in the three with negative result was so dry as to be quite brittle, while in the fourth it was noticeably much softer in consistency. The presence of living organisms in the gum exuded from a dead leaf would seem to discount the hypothesis of active bacteriolysis taking place within this medium.

The foregoing experiments emphasised the difficulty of isolating the parasite, but failed to determine the percentage of spots containing viable bacteria. The number of cases where *P. Proteamaculans* appeared was very few, but it was impossible to say how far the sterilisation of the leaf surface had contributed to this result. Experiments with unsterilised leaves were equally unsuccessful through the difficulty in recognising the parasite in the presence of the numerous other organisms which developed. One of these, a small *Sarcina*, very closely resembled diplo-forms of the *Pseudomonas*.

Experiments were next made to determine the fate of bacteria in spots produced by artificial infection. The surface of a fresh leaf of a young seedling of *Protea cynaroïdes* was first washed with 60 per cent. alcohol, scratched in several places with a sterile needle and the scratches covered with uniform drops of a bouillon culture of *P. Proteamaculans*. On the sixth day the leaf was washed with sterile water to remove organisms from the surface. The first spot was cut out on this day and the others at intervals of every second or third day, and the number of bacteria counted. The spots were cut out with a sterile cork-borer, ground in 5 c.c. of sterile water with 1 gm. of sterile sand, suitable dilution plates

were made in bouillon agar and the colonies counted after two days' incubation[1]. Fig. 1 shows the result of this experiment. It will be seen that the numbers rose from 1,242,000 on the sixth day to 2,808,000 on the fifteenth day, and then fell rapidly to 16,000 on the thirty-second day, when the experiment came to an end. This experiment was made in July. A repetition was made in October of the same year, drops of a suspension of the organism in sterile water being used instead of a bouillon culture. This series was very unsatisfactory in the number of successful inoculations; out of 24 spots only the following five records were

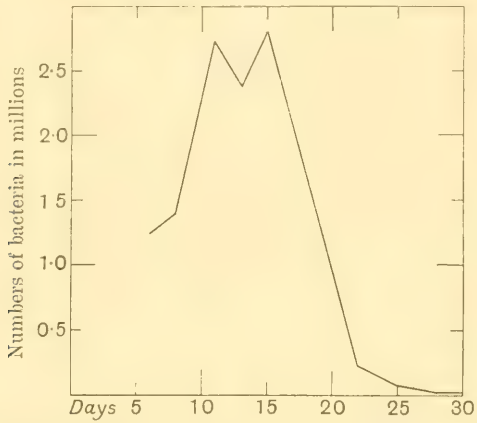

Fig. 1. Showing the rise and fall in the numbers of bacteria in leaf spots artificially infected with *Pseudomonas Proteamaculans*.

obtained: after 9 days, 1,280,000; 15 days, 1,576,000; 27 days, 1,808,000; 35 days, 1,336,000; 40 days, 485,000.

A sixth spot showed an exuded drop of mucilage which has been regarded as the first sign of active infection only on the forty-third day and gave a count of 1,850,000. A seventh spot developed actively some time later.

These experiments showed clearly that the rate of destruction of the organism was not very rapid, and again negatived the hypothesis of the production of a bacteriolysin by the invaded cells.

[1] The degree of accuracy of this method was tested in the following manner: First, as to the uniformity of the individual drops of bacterial suspension applied to the leaf—nine drops were plated separately and counts made after two days' incubation varied from 601 to 746. Second, as to the number of bacteria lost by adsorption on the sand and ground-up tissue—four experiments were made comparing the numbers in drops of suspension before and after grinding with sand and sterile leaf tissue: in one of these the loss was 20 per cent. and in the other three 9 per cent.

The question of auto-intoxication of the bacteria was next considered. A broth culture of the organism was placed out in uniform drops upon small squares of sterile mica and maintained in a moist Petri dish. Periodical counts of the organisms were made in bouillon agar after suitable dilution in sterile water. The numbers of living bacteria remained about 3–4 millions for five weeks (disregarding one low count of 1,760,000) and at the end of eight weeks, when the experiment was concluded, they still amounted to nearly one million. The falling off of the numbers in the infection experiments, which became marked in 30 days and 40 days in July and October respectively, was therefore not due to auto-intoxication of the invading organism.

The resistance of the organism to desiccation was next investigated. Two measured drops of a broth culture were placed on squares of sterile mica. One was immediately washed off with sterile water and plated. The number of organisms present was determined as 1,328,000. The other drop was allowed to dry for 24 hours and was then washed and rubbed off the mica with a sterile glass rod. The numbers of bacteria were found to have fallen to 11,000. In a second experiment the numbers were reduced from 890,000 to 15,000 in 24 hours, to 6400 in four days and to zero in eight days.

In order to approach more nearly the conditions of nature the experiment was repeated using in the place of the mica small pieces of *Protea* leaf which had been dried in air after having been heated in the autoclave. A water suspension containing some four million organisms per drop was employed and the result was as follows: after 1 day, 2,044,340; 2 days, 569,050; 3 days, 7069; 6 days, 7214; 9 days, 802; 13 days, 320.

Obviously desiccation is an important factor and no doubt accounts for the death of the organism in the diseased tissue. The non-viability in the dry gum and viability in moist gum as described on p. 22, is explained in this way, as also is the relatively slower onset of sterility in the October infection experiment as compared with the July experiment described above, drying presumably being more rapid in July.

Under favourable conditions the moisture content of the invaded tissue remains sufficiently high to maintain the life of the organism for some considerable time. The organism has been re-isolated from artificially infected spots 65 and 75 days after infection. Even after the lapse of 16 weeks in one case two colonies of *P. Proteamaculans* were obtained on grinding the diseased spot and plating.

The rapid growth of the organism and the area of the spots produced

are evidently restricted by cork formation and production of wound gum, and the ultimate death of the organism is the result of desiccation within the area enclosed by the cork.

Microscopical examination of the tissues.

The fact that the gummy material present in the spots is softened and slowly dissolved by chromic acid has rendered it possible to get fair microtome sections varying from 4–6μ in thickness after the use of chromic acid fixatives. The most satisfactory stain for the bacteria in the tissues was found to be carbol fuchsin followed by light-green in clove oil.

Sections through an artificially produced spot seven days after infection show masses of a small diplo-bacillus with thick capsule lining the cavity produced by the infecting needle and extending into the neighbouring cells and intercellular spaces. The shrivelled cells surrounding the cavity contain clear yellow gum, those beyond a paler vacuolate gum, mostly crammed with bacteria, but in certain cases quite clear and structureless. The view that the granular appearance of the gum in many cells is due to the inclusion of bacteria is confirmed by these sections, and also by the fact that the gum exuded by the dead leaves contains the diplo-bacillus, but in a smaller form than that obtained from an agar culture. The strong affinity of this granular gum for bacterial stains such as carbol fuchsin is thus accounted for.

The infected spots are sometimes, but not always, cut off by a definite layer of cork; multiplication of the host cells has usually occurred and their walls do not give the cellulose reaction with iodine and sulphuric acid; they and the gum in the intercellular spaces stain red with phloroglucin, possibly owing to the presence of wound gum.

The clear structureless gum which accumulates in the cells bordering the spot, mainly in the palisade parenchyma, before the plastids and cell contents are disorganised, is coloured rose-red in fresh hand sections, and on treatment with normal caustic soda turns a bright chlorophyll green. In microtome sections where it is pale and colourless it stains green with chlorophyll extract and yellow with potash, which seems to indicate that it contains something allied to suberin.

These observations show that the host plant has considerable powers of resisting the invasion of the parasite by producing cork, the strict localisation of the disease being thereby accounted for.

Summary.

1. The production by the cells of *Protea cynaroïdes* of a bacteriolysin against the organism which produces a leaf-spot disease, suggested in a former paper, has not been confirmed.

2. The bacteria enter the leaf by way of the stomata; the local lesions produced are prevented from spreading by the development of wound cork.

3. The causal organism succumbs very readily to the desiccation to which it is subjected owing to the cells which it attacks being cut off by the wound cork from their water supply.

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THE KILLING OF BOTRYTIS SPORES BY PHENOL[1]

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(With 11 Text-figures.)

Several workers have studied the manner in which bacteria are killed when submitted to the action of poisons: their conclusions are referred to later. One might expect that in the case of fungi the findings would be the same, but little accurate and detailed information is available, and as there is still a great deal of uncertainty, partly as to the facts, and still more as to their interpretation, in the killing of bacteria, it seemed possible some further light might be obtained by a study of the problem, using fungi as the test object.

The organism selected was *Botrytis cinerea*, the strain used being the one described by Brierley (1). From a single spore of this strain cultures were obtained on Czapek's medium, and in all the experiments detailed here Czapek's has been the only medium used.

Method.

The method adopted was as follows. The spores were sown on Czapek-agar slopes, which were kept at room temperature in a subdued light till used. From these a suspension of spores was prepared of the desired density in distilled water. In removing the spores care was taken not to touch the subjacent medium and to obtain a suspension as free as possible from hyphae. This was done by laying lightly on the tops of the conidiophores a platinum loop filled with distilled water: the spores adhere to the film of water on the loop and are transferred to a tube of water and washed off. By repeated application of the loop a suspension is obtained of practically pure spores. To a measured volume of the suspension, previously brought to the desired temperature, was added an equal volume (also previously warmed) of the toxic agent to be examined, in such strength that the final mixture was of the desired concentration. The mixture was maintained in a water-bath at

[1] A grant in aid of publication has been received for this communication.

the temperature indicated in each experiment, and kept stirred; and samples were withdrawn at intervals for examination. The volume of the mixture was not less than 10 c.c., usually about 20 c.c., and the samples were of 0·5 c.c. volume, except when the suspension was very dense, in which case smaller samples were taken. Each sample was immediately dropped into a centrifuge-tube containing 8 c.c. of distilled water (in experiments with high concentrations of phenol 10·5 c.c. were used), and rapidly centrifugalised. The supernatant fluid was removed till about 0·25 c.c. remained, and in this the deposited spores were stirred up to form a suspension. From the latter a standard platinum loopful was transferred to a coverslip, on which had been placed a standard loopful of Czapek's fluid, and the two drops well mixed. The coverslip was then placed on a van Tieghem cell, and this incubated at 26° C. for 22 to 24 hours. Two to four such cells were prepared from each sample. After incubation they were examined with a Zeiss C objective and No. 6 eyepiece, and the proportion of spores that had germinated ascertained by counting successive fields. This is readily done, the spores capable of germination producing in the time allowed hyphae or germination tubes which are easily seen. As a rule, the whole drop was counted, and to get reliable figures it is necessary to count several hundred spores from each sample. Four or five hundred spores are enough, but in most cases six or eight hundred, and sometimes more, were counted. It is sometimes stated that more spores germinate round the edges of the drop than in the centre(2). I was unable to convince myself that this occurred under the favourable germination conditions used in these experiments, but it is advisable to count all spores in the whole drop, in order to avoid any error from this source.

The method gives uniform and satisfactory results. The first experiment given below is set out in detail to illustrate the amount of variation obtained in duplicate slides. The variation does not exceed the standard error of sampling. But to get consistency the technique must be carried out with rigid uniformity and scrupulous attention to details. All slides, pipettes, coverslips, etc. are, of course, cleaned and sterilised. There would seem to be great opportunity for contamination in mixing the drops on the slips, but as a matter of experience contamination is a rare occurrence—I estimate it at less than 2 per cent. of all preparations made. The bottom of the van Tieghem cell (of which the rings were fixed with beeswax) was covered with Czapek solution, the coverslip laid on without vaseline or other cement, and the whole cell placed in a covered glass dish containing distilled water to prevent drying. In

cases where it was impossible to complete the counts quickly enough, a few drops of chloroform were added to the cell after incubation. This arrested all further growth and the slides could be counted at leisure. Some device of this sort was necessary, because otherwise the hyphal growth becomes so profuse as to make accurate counting impossible.

The dilution of the sample in the centrifuge tube arrests the action of the phenol by lowering its concentration to a point much below that which has any perceptible effect on the spores in the time given. The number of spores contained in the final mixtures was determined by direct counting in the Thoma-Zeiss haemocytometer.

In Table I is given the result of an experiment with 0·4 per cent. phenol and spores taken from the tube 27 days after inoculation. During the first 20 minutes apparently none of the spores are killed, but in the second 20 minutes about 1·5 per cent. die, and in the third 20 minutes a further 7·5 per cent., in each successive interval more and more dying

Table I. Expt. of 23. 10. 19. Spores 27 days old; 150,000 per 1 c.c. Phenol 0·4 per cent. Temp. 25°.

Sample taken in minutes	Slide No.	Number counted	Per cent. germinated	Total number	Per cent. germinated	Proportion
0	1	353	91·5	590	91·5	100
	2	237	91·5			
30	1	300	91·0	651	92·0	100·5
	2	351	92·8			
50	1	353	87·2	579	85·3	92·9
	2	226	82·5			
70	1	541	79·1	973	76·0	82·8
	2	432	72·2			
80	1	142	61·2	573	60·5	65·9
	2	431	60·3			
90	1	453	48·9	887	50·8	55·3
	2	434	52·7			
100	1	359	35·3	767	35·2	38·3
	2	408	35·0			
110	1	601	26·4	1011	24·2	26·3
	2	410	20·9			
120	1	262	24·4	585	22·3	24·2
	2	323	20·7			
130	1	446	19·2	814	15·4	16·7
	2	368	10·8			
140	1	292	6·16	655	6·71	7·31
	2	363	7·27			
150	1	350	3·7	484	4·95	5·39
	2	134	8·2			
160	1	442	2·03	808	2·84	3·09
	2	366	3·82			
170	1	540	1·85	827	1·69	1·84
	2	287	1·39			

until the period of 80–100 minutes is reached. During this interval nearly 30 per cent. of the total number of spores are killed. Thereafter fewer and fewer are killed in each time-interval, until in the 160–180 minutes interval only about 1 per cent. die; and the last survivor would not succumb until some four hours or more had elapsed from the beginning of the experiment. The whole curve, which may be called a survivor curve, has a sigmoid shape (see Fig. 1).

In Tables II, III and IV are given experiments with higher amounts of phenol, viz. 0·5, 0·6 and 0·7 per cent., and three of these are also plotted in Fig. 1. The close scale of the figure somewhat obscures the character of the curves with the higher strengths, but if plotted on a more open scale they will be seen to conform to the general type of the 0·4 and 0·5 per cent. curves.

Table II. 18. 9. 19. 27 day spores. 200,000. 25·2°. Phenol 0·5 per cent. Control: 89·5 per cent. of 777 spores germinated.

Sample taken in mins.	Number counted	Per cent. germinated
0	777	100
20	683	94·6
40	735	73·1
60	913	28·9
80	1135	8·37
100	1066	2·3
120	744	1·9
140	1465	0·76
160	1248	0·71

Table III. 30. 10. 19. Phenol 0·6 per cent. 34 day spores. 280,000. Control: 83·88 per cent. germinated in 515. 24·8°.

Sample taken in mins.	Number counted	Per cent. germinated
0	515	100
3	289	95·3
6	541	93·3
9	757	76·6
12	456	66·6
15	357	45·3
18	885	16·8
21	694	13·8
24	637	2·6
27	835	0·84
30	1514	0·39

After 39 mins. two spores only germinated; after 42, 45 and 48 mins. none; after 51 mins. one; and after 54 mins. none.

Table IV. 1. 10. 19. Phenol 0·7 per cent. 18 day spores. 25°. Control: 86·95 per cent. of 914 spores germinated.

Sample taken in mins.	Number counted	Per cent. germinated
0	914	100
1.5	1503	71·3
2.5	632	52·1
3.5	1143	36·0
4.8	524	19·2
8.0	1195	2·04
11.25	1125	1·57

These experiments have been frequently repeated and always with the same general result. In experiments performed on different days and with different spore cultures the minor details never completely correspond, and it is almost impossible at a later date to reproduce exactly the result obtained on a previous occasion. For this many factors are responsible. The age of the culture and the number of spores used are of great importance, as will appear later, but the chief source of the discrepancies lies in the almost insuperable difficulty of obtaining on different days suspensions of spores which are perfectly alike. Small differences in the degree of moisture in the culture tubes, the tightness or looseness of the cotton wool plugs affecting the oxygen supply, and

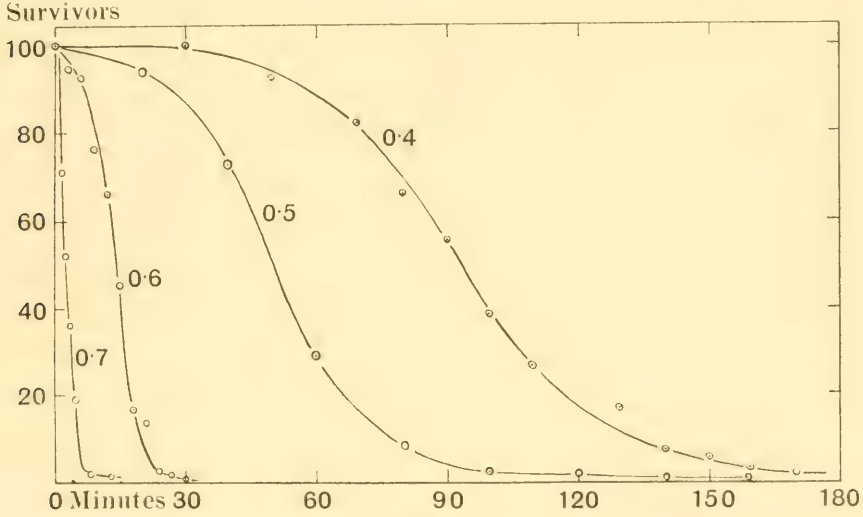

Fig. 1.

the like, modify the rate and character of the development of the spores, and this is reflected in the mortality rate. But the general character of the curves remains the same, a sigmoid curve with a stage of increasing steepness, a maximum, and finally a stage of decreasing steepness, flattening out more and more as time goes on.

This is unlike the curve generally accepted as the typical mortality curve for bacteria exposed to disinfectant agents, such as phenol, corrosive sublimate, heat, sunlight, etc. Since the papers of Chick in England(3, 4) and Madsen and Nyman in Denmark(5), the typical mortality curve has been recognised as a logarithmic one, corresponding in shape to the unimolecular curve, in which the number dying at any

moment is strictly proportional to the number alive at that moment. These workers do not as a matter of fact always obtain curves of this type—many of their results indeed depart very widely from it. But the logarithmic type is held to be the true one, and the departures from it to be variations caused by factors independent of the disinfection process itself. The sigmoid shape of curve is, on the other hand, a type occurring with great frequency in biological investigations of this kind. The literature of haemolysis is full of examples of it. Even with bacteria some workers find curves of this type occurring with heat or chemical poisons, and some of the experiments with staphylococci recorded by Chick would seem to fit a sigmoid curve as reasonably as a logarithmic one. It occurs also with more highly developed organisms, *e.g.* it was obtained by Boycott in the killing of tadpoles by hot water(6). A good example may be seen in a recently published paper on the habits of the Tomato Moth(7), where the poison used was a lead arsenate paste. The plants were sprayed with a suspension of the paste, and then infected with the larvae of the tomato moth. On successive days thereafter the survivors were counted, and the results are tabulated. It is found that the larvae die off gradually, some being dead the day after the spraying, others surviving a week or more. The explanation offered is that the larvae find the paste distasteful, and the majority cease feeding before they have taken a fatal dose. After a time hunger forces them to recommence feeding, until finally a fatal dose is absorbed by all. The death-rate, however, is probably an example of the same phenomenon as that described above. I have plotted the figures given by Lloyd and reproduce in curve form two of his experiments (see Fig. 2). The sigmoid curve is well illustrated and presents the same general features as the curves obtained with Botrytis spores.

It is not difficult to find an explanation of the peculiar shape of the sigmoid curve. We do not know what is the nature of the reaction occurring between the phenol and the spores, whether, for example, it is a chemical combination of the phenol with the protoplasm of the spore, or a physical partition of the phenol between the two solvents protoplasm and water, resulting in the coagulation and death of the spore (cf. Cooper(8, 9)). We are equally ignorant of the nature of the resistance offered by the spores to the poison. It is, however, reasonable to assume that in a large number of organisms, such as those in the suspensions of Botrytis spores, the individuals will differ from one another in resistance, and that if we graduate the resistances and determine the number of individuals in each grade (construct, in fact, a

frequency table), and then plot the results graphically, the curve so obtained will conform more or less closely to the normal curve. This is in accordance with a large mass of biological experience, both anatomical and physiological; and is likely to be true, whatever may be the nature of the resistance offered—whether, *e.g.* it has a structural basis, such as thickness or composition of the spore coat, or depends on some such property as a capacity to neutralise or destroy the phenol as it enters the spore. If this be granted, then the sigmoid survivor curve is what we should expect to find. For in the mass of spores exposed to the phenol those will die first which have the lowest grade of resistance: and these are few in number. In each successive time-interval further grades of resistance will be overcome, and these grades

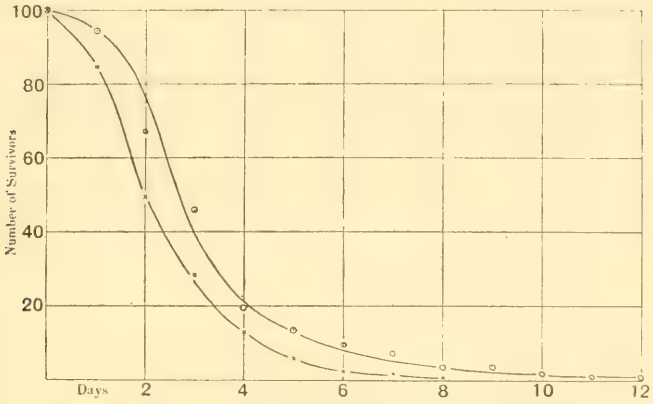

Fig. 2. Tomato moth larvae and lead arsenate spray (Lloyd).
⊙——⊙ 3 lbs. per gallon. ×——× 6 lbs. per gallon.

contain more and more individuals, so that in the successive intervals more and more spores will die: until the middle grade of resistance is passed. After that, in successive intervals more and more grades of resistance will be overcome, but as the individuals in these grades are less and less in number, fewer and fewer spores will die, until eventually only a very few individuals are left in each remaining grade. A curve expressing the numbers left alive after each time-interval, *i.e.* a survivor curve, will have a symmetrical sigmoid shape, and will resemble the survivor curve we obtain with 0·4 per cent. phenol, shown in Fig. 1.

The idea of explaining the different life-times of the spores by variations in the resistance of the individual spores was put forward at least as long ago as 1889 (16), and if we assume the variations to conform

to the normal curve, it gives an adequate and satisfactory explanation of the sigmoid curve.

If, however, we examine more closely the curves obtained with 0·5, 0·6 and 0·7 per cent. phenol, it becomes apparent that their shape, though still sigmoid in character, differs from that of the 0·4 per cent. curve, the difference being progressively more marked as the strength of phenol is increased. All the experiments recorded in Tables I—IV were done with adult spores in approximately equal numbers and at nearly the same temperatures. They may fairly be compared; and in the following table (V) the results of these and other[1] similar experiments done with each strength of phenol are brought together. The curves were drawn for each experiment, and the times required to kill 25, 50 and 75 per cent. of the spores read off, and from these readings the mean times for each strength of phenol obtained.

Table V. Times in minutes taken to kill 25, 50 and 75 per cent. spores by 0·4, 0·5, 0·6 and 0·7 per cent. phenol. In brackets are given the figures for each with the 0·7 per cent. times taken as unity.

Phenol per cent.	25 per cent.	50 per cent.	75 per cent.
0·4	90·25 (34·8)	103 (24·5)	118·5 (18·9)
0·5	34·50 (13·2)	45 (10·7)	53·5 (8·5)
0·6	9·12 (3·5)	11·23 (2·6)	13·48 (2·1)
0·7	2·6 (1·0)	4·2 (1·0)	6·25 (1·0)

It will be seen that the times required fall rapidly as the strength of the phenol is increased—*e.g.* while 90 minutes are required with 0·4 per cent. to kill 25 of each 100 spores, with 0·7 per cent. only 2·6 minutes are necessary. But it is also apparent that the ratios of the times required vary greatly, if we compare the 25 per cent. with the 50 and 75 per cent. columns. It takes 34 times as long for 0·4 per cent. phenol to kill 25 per cent. of the spores as for 0·7 per cent. to kill the same number: but it takes only 19 times as long for 0·4 per cent. to kill 75 per cent. as for 0·7 per cent. With the strong poison the time of killing is much more reduced in the early part of the process than it is in the later stages. The effect of increasing the quantity of phenol is to accelerate the killing much more markedly in the early part of the curve—*i.e.* increasing the phenol tends to remove the slow stage at the beginning of the curve and to reduce the sigmoid character: the curve tends more and more to approach the logarithmic type as the phenol is more and more raised.

[1] The cost of printing at present prevents these being given in full.

If we write the last table in a different form, taking as 1 the time required to kill 75 per cent. of the spores, we get Table VI.

Table VI.

Per cent.	25 per cent.	50 per cent.	75 per cent.
0·4	0·76	0·87	1·0
0·5	0·64	0·84	1·0
0·6	0·67	0·83	1·0
0·7	0·41	0·67	1·0
Unimolec. curve	0·20	0·50	1·0

The corresponding time-ratios for a unimolecular curve are added to this table, and it will be seen that the observed figures approximate more and more closely to the logarithmic type, the greater the strength of phenol used.

It is unfortunately not practicable to use higher concentrations of phenol at this temperature, 25° C. With 0·8 and 1·0 per cent. the reaction runs so fast in the early stages that it becomes impossible accurately to sample quickly enough. In 0·8 per cent. phenol, for example, 43·27 per cent. survive for 30 seconds, 5·7 per cent. for 1 minute, 3·6 per cent. for 1·5 minutes, and 0·39 per cent. for 2·5 minutes. Already in half a minute the number of survivors falls from 100 to 43, and it is impossible to observe the important early part of the curve.

Effect of Low Temperatures. An attempt was therefore made to work at much lower temperatures, in the hope of so slowing the reaction as to make observation practicable. All apparatus, the phenol solution, the suspension of spores, etc., were previously cooled to the temperature of melting ice, from 1·5° to 2·0° C., and the samples dropped into ice-cold water and centrifuged as quickly as possible. Control experiments showed that germination was not altered in numbers by the exposure to cold, but was rendered a little slower during the subsequent incubation. With 2·0 per cent. phenol at 2·0° all spores were killed in 20 seconds. With 1·5 per cent. phenol at the same temperature 1·32 per cent. survived 21 seconds, and 0·52 per cent. survived 41 seconds.

At the low temperatures it was possible to observe fairly satisfactorily the course of the reaction with 1 per cent. phenol. With higher concentrations the process was too rapid for detailed examination (see Tables VII–XI). It is apparent, however, that with 1·25 per cent. the sigmoid character is still present: and with 1 per cent., whether at 1·5° or 7–8°, the curve is frankly sigmoid (*v.* Fig. 3), as markedly as with 0·7 per cent. at 25°, even although the reaction proceeds more quickly. These results will be referred to later (*v.* p. 49).

Table VII. 24. 8. 20. 2° C. Phenol 1·31 per cent. 25 day spores. 290,000. Control: 94·15 per cent. germinated.

Time in seconds	Number counted	Per cent. germinated
0	855	100
20	927	8·69
40	1144	0·46
62	1217	0·43
120	533	0·39

Table VIII. 20. 8. 20. 2° C. Phenol 1·25 per cent. 21 day spores. 175,000. Control: 92 per cent. germinated.

Time in seconds	Number counted	Per cent. germinated
0	882	100
20	1117	47·8
40	513	1·9
60	687	0·31
92	1476	0·44

Table IX. 17. 8. 20. 1·5° C. Phenol 1·0 per cent. 18 day spores. 175,000. Control: 81·7 per cent. germinated.

Time in seconds	Number counted	Per cent. germinated
0	1422	100
21	988	68·4
41	619	94·5
61	508	90·2
90	400	82·6
123	464	53·5
154	488	27·8
180	524	17·6
212	499	5·14
250	710	2·92
300	934	1·30

Table X. 5. 8. 20. Phenol 1·0 per cent. 7–8° C. 37 day spores. 500,000. Control: 97·45 per cent. germinated.

Time in seconds	Number counted	Per cent. germinated
0	1334	100
0·5	1192	62·75
1·25	1794	10·98
2	1756	3·86
3·5	1690	1·33
4·5	1953	0·77

Table XI. 5. 4. 20. 12° C. Phenol 0·8 per cent. 23 day spores. 200,000. Control: 88·729 per cent. germinated.

Time in minutes	Number counted	Per cent. germinated
0	1568	100
1	860	93·96
2·25	622	94·39
4	828	88·08
6	576	67·89
8	1760	52·37
10	1715	26·74
13	1137	15·55
16	1208	5·12
19	1563	2·59
24	1399	1·69

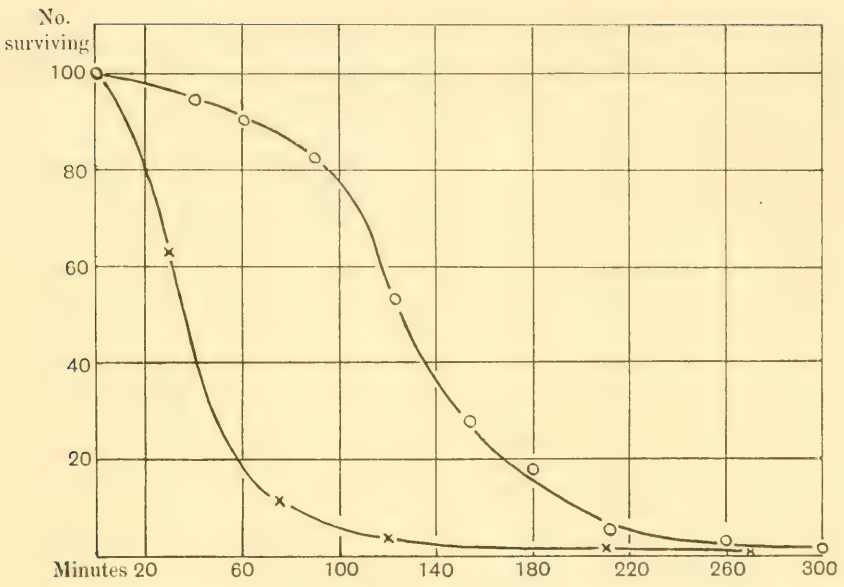

Fig. 3. Phenol 1 per cent.
○——○ Temp. 1·5° C. ×——× Temp. 7–8° C.

Effect of Varying Number of Spores. In Tables XII–XV are given a series of experiments, in each pair of which one and the same strength of phenol was used with suspensions of different density. Each pair was done at the same time with spores from the same suspension suitably diluted: and the results are strictly comparable with one another.

Table XII. 15. 12. 19. Phenol 0·4 per cent. 58 day spores. 25·2°.

Time in minutes	1,000,000 spores in 1 c.c.	11,400 spores in 1 c.c.
0	100	100
10	—	96·5
30	96·4	92·0
50	—	92·1
60	97·1	86·1
70	—	79·4
80	91·4	64·3
90	86·8	62·7
100·5	83·5	—
132·5	—	15·3
140	71·0	—

Table XIII. 8. 12. 19. Phenol 0·4 per cent. 51 day spores. 25°.

Time in minutes	2,700,000 spores in 1 c.c.	5000 spores in 1 c.c.
0	99·6	100
10	100·1	90·4
20	100·7	82·8
30	100·1	—
40	100·3	57·1
50	98·9	—
61	100	—
	Mean 100·0	

Table XIV. 26. 11. 19. Phenol 0·6 per cent. 50 day spores. 25°.

Time in minutes	400,000 spores in 1 c.c.	50,000 spores in 1 c.c.
0	100	100
5	90·1	43·7
10	27·0	16·3
15	9·32	4·50
20	2·08	—
25	0·99	0·96
30·5	0·31	—
35	0·185	—

Table XV. 17. 12. 19. Phenol 0·7 per cent. 50 day spores. 25·2°.

Time in minutes	3,000,000 spores in 1 c.c.	7000 spores in 1 c.c.
0	100	100
1	99·8	98·5
2	94·2	79·3
3	94·4	66·1
4	88·7	61·1
5	84·9	28·4
6	82·9	12·0
7	—	6·5
8	49·8	—
8·5	—	2·6
10	—	1·1
12	11·9	—
14	—	0·56

Table XVI. 2. 12. 19. Phenol 0·7 per cent. 45 day spores. 25·0°.

Time in minutes	1,200,000 spores in 1 c.c.	Time in minutes	32,000 spores in 1 c.c.
0	100	0	100
1·25	95·4	1·25	98·2
2·5	86·5	2·25	71·5
3·5	57·8	3·25	54·0
4·5	39·5	4·25	34·02
5·5	19·1	5·25	7·27
6·5	15·8	6·25	4·60
9·5	1·71	9·25	0·58

In Fig. 4 are charted the results of four experiments with 0·4 per cent. phenol and spores which varied in numbers from 5000 to 2,700,000 in each c.c. of the killing mixture. If we plotted on the same figure the curve already shown in Fig. 1, where 150,000 spores were used, its graph would come between the 11,400 and the 1,000,000 curves. It is evident that the increase in the number of spores has greatly slowed the rate of killing. With the largest number all are still alive after one hour. If this experiment had been continued I do not doubt that the curve would have eventually begun to drop like the others. The same thing is seen in the 0·6 and 0·7 per cent. experiments. The larger the number of spores employed, the more marked is the delay in the early part of the curve.

The bearing of these facts on infection is evident. It is well known that the larger the size of the inoculum the greater is the chance of a

successful inoculation being obtained, and that even fully virulent organisms may fail to produce infection when given in a small dose.

It is perhaps rather surprising that the change in the number of spores present should make so much difference in the times required to kill. The average dimensions of the spores used are about 10 μ long by about 8 μ broad. Each spore certainly weighs less than a piece of flint of the same size would weigh. But if we assume that the spore is a sphere 10 μ in diameter and that each spore has a weight of the same

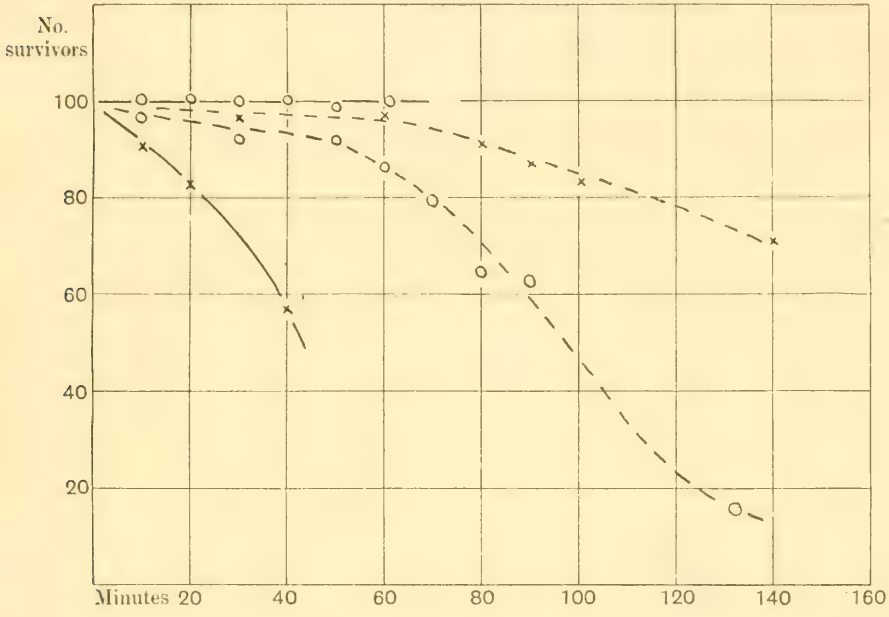

Fig. 4. Phenol 0·4 per cent.

—○—○ 2,700,000 spores in 1 c.c. - - × - - × 1,000,000 spores in 1 c.c.
- - ○ - - ○ 11,400 spores in 1 c.c. —×—× 5000 spores in 1 c.c.

order as flint, viz. three times that of water—and this is greatly to exaggerate the weight of the spores—we have in a 0·4 per cent. phenol mixture, containing 1,000,000 spores in each 1 c.c., two and a half times as much weight of phenol as of spores. In a 0·7 per cent. mixture with the same number of spores there is present four times as much weight of phenol as of spores. We cannot suppose that the individual spore will take up nearly its own weight of phenol. I have not yet succeeded in estimating directly the amount of phenol actually removed from such solutions by the spores, for the quantities concerned are very minute,

and the disturbing influences difficult to eliminate. It seems probable, however, that the individual spore does not remove one-hundredth part of its own weight of phenol. If this is so, one might expect that a solution containing by weight several hundred times the amount of phenol taken by the spores would contain a practically infinite amount of phenol relatively to the spores, and that any alteration of the phenol-spore ratio would have a scarcely appreciable effect on the time of killing. But this, as we have seen, is not in fact the case.

Experiments with Young Spores. We have seen that for technical reasons it was not possible to obtain a logarithmic survivor curve when using adult spores, and that the use of low temperatures did not help. It is, however, possible to approach the question in another way by using spores of a different character. At room temperature on Czapek-agar Botrytis spores are first clearly visible to the naked eye on the 5th to the 6th day after inoculation of the tube. When first formed they are in the mass of a pale grey colour instead of the dark mouse colour they assume later. They are at this period already "ripe," since they are fully formed and germinate freely, but they are less resistant, as may be seen on a comparison of the times required for killing in the following tables with those already given. I accordingly carried out a series of experiments with young spores removed from the tubes soon after they were clearly formed. These are detailed in Tables XVII–XIX, and the graphs drawn in Figs. 5 and 6.

Table XVII. 22. 3. 20. 9 day spores. Phenol 0·7 per cent. Both series from same suspension. 26·3°.

Time in minutes	30,000 in 1 c.c.	240,000 in 1 c.c.
0	100	100
0·5	89·46	97·21
1	82·06	79·56
2	76·23	71·60
3	60·87	47·17
4	44·28	28·80
5	30·60	10·86
6	14·23	7·30
8	3·37	1·27
10	0·79	0·52
12	0·66	—
23	0·28	0·15

Table XVIII. 1. 4. 20. 7 day spores. Phenol 0·7 per cent. 26·2°.

Time in minutes	40,000 in 1 c.c.	Value of k
0	100	—
0·5	82·51	0·167
1	64·08	0·193
2	52·94	0·138
3	35·61	0·149
5	13·04	0·176
6	8·93	0·175
7	1·85	0·247
8	1·15	0·242
9	0·19	0·302
11	0·0	—

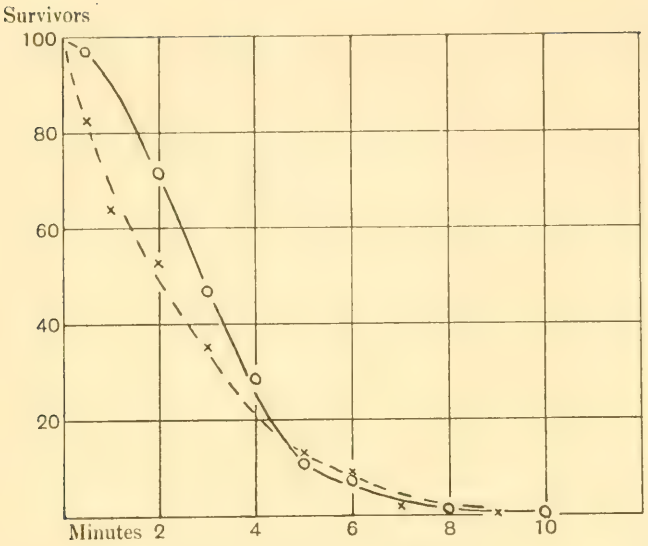

Fig. 5. Phenol 0·7 per cent.
○—○ 9 day spores. × – – × 7 day spores.

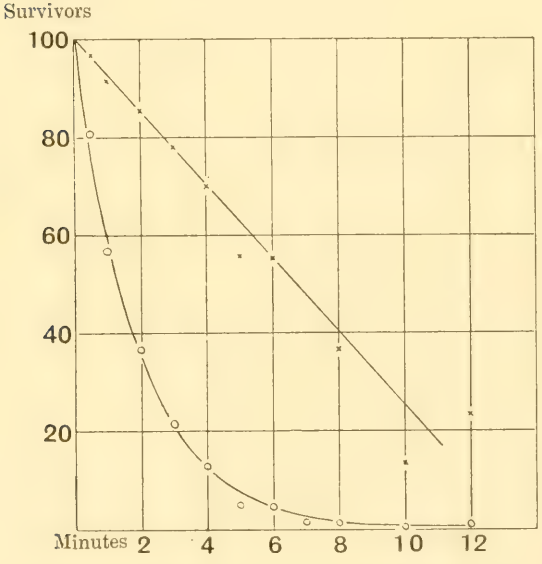

Fig. 6. Phenol 0·7 per cent. and 6 day spores.
The curve is drawn to the formula with $k=0{\cdot}226$; the circles are the observed points. The crosses represent the logarithms (to base 10) of the observed number of survivors plotted against time.

Table XIX. 12. 4. 20. 6 day spores. Phenol 0·7 per cent. 25·2°.

Time in minutes	60,000 in 1 c.c.	Value of k
0	100	—
0·5	81·09	0·182
1	56·67	0·246
2	36·73	0·217
3	21·50	0·222
4	12·62	0·224
5	4·66	0·262
6	4·47	0·228
7	1·32	0·268
8	1·18	0·241
10	0·25	0·260
12	0·55	0·188
		Mean 0·230

With 9 day spores the sigmoid character is clear, more marked with 240,000 than with 30,000 spores to the 1 c.c. With 7 day spores, however, the character is altered. There is no longer perceptible to the eye an initial period of increasing rate and the curve falls steeply from the start and then slows off as before. It approaches much more closely to the logarithmic type than any of the preceding curves (*v.* Fig. 5). In the table (XVIII) is added the values of k on the assumption that the process follows the unimolecular formula $k = 1/t \log a/a - x$, where a is the original number of germinable spores and x the number dead at time t. The constancy of k is not so good as one would hope for, even in this type of work, and rises definitely towards the end. With 6 day spores, however (the youngest spores which it was found practicable to use), the value of k is as constant as one can reasonably expect, at least until only about 4 per cent. of the spores are still surviving (*v.* Table XIX). In Fig. 6 the curve is drawn from the formula with $k = 0{\cdot}226$, and the observed points fall very closely on the curve. In Fig. 6 also the logarithms of the observed values are plotted against time, and the points from 100 per cent. to under 10 per cent. fall very fairly on a straight line.

With sufficiently young spores, then, the curve is of the logarithmic type, and the sigmoid character has disappeared. It was of interest to determine whether with a low strength of phenol it would be possible to restore the sigmoid shape, when using spores which gave the logarithmic type with the higher strength. An experiment was accordingly performed with 6 day spores and two strengths of phenol. The same suspension was used in both cases, one-half being exposed to 0·7 phenol.

and the other half to 0·4 per cent. phenol, and the experiments were carried out simultaneously. The result is seen in Table XX and Fig. 7. The 0·7 per cent. curve shows no sigmoid character and approaches closely the unimolecular type, although in this particular experiment not strictly logarithmic (*v.* values of k in the table). The 0·4 per cent. curve, on the other hand, is frankly sigmoid. The same spores, then, give a sigmoid or a logarithmic curve according to the strength of phenol employed. The shape of the curve does not depend simply on the character of the spores used, but chiefly on the strength of phenol.

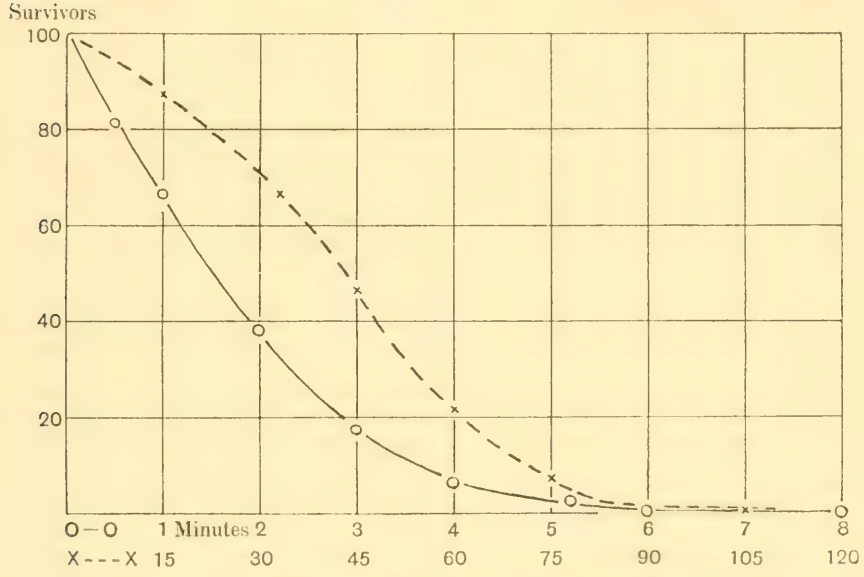

Fig. 7. Same suspension with × --- × 0·4 per cent. phenol and
○ —— ○ 0·7 per cent. phenol.
The 0·7 per cent. graph is plotted on a 15 times more open scale than the 0·4 per cent.

What, then, is the explanation of the logarithmic type of curve? It is evident that the explanation given for the sigmoid 0·4 per cent. curve will not serve here, at least not without modification. The 0·4 per cent. curve, when transposed into a frequency curve in the manner described, gives an approximation to a normal curve (*v.* Fig. 8), and the explanation derives its convincing cogency from the fact that the derived frequency curve approaches the normal curve so closely. In Fig. 8 is also drawn a strictly normal curve ($\log y/16 = -x/3113$). A better fit could no doubt be obtained by suitable adjustment, but it

Table XX. 20. 4. 20. 6 day spores. Phenol 0·4 and 0·7 per cent. with same suspension. 60,000 spores in 1 c.c. 25·6° C.

0·4 per cent.		0·7 per cent.		
Time in minutes	Per cent. germinated	Time in minutes	Per cent. germinated	Value of k
0	100	0	100	—
15	87·62	0·5	81·5	0·176
33	66·27	1	66·5	0·177
45	46·33	2	38·17	0·209
60	21·82	3	17·61	0·251
75	7·36	4	6·24	0·301
90	0·63	5·2	2·22	0·317
105	0·166	6	0·12	0·420
120	0·0	8	0·0	—
135	0·0			

will be seen that the experimental curve is not far removed from the normal. But if the 0·5, 0·6 and 0·7 per cent. curves are similarly transposed, the resulting frequency curves do not resemble normal curves. They are skew, the modal value has moved to the left, and moves further and further to the left, the greater the strength of phenol used (*v.* Fig. 9). If a logarithmic survivor curve were similarly transposed, it would give a "frequency curve," which has somewhat the shape of a reversed J, with the modal value on the extreme left.

This extreme J-form is a very unlikely form of distribution to find in an assemblage of Botrytis spores. Biological frequencies of this type are, of course, known, but they are very uncommon and we should not expect to find them here. This distribution is so unusual that those observers who regard the logarithmic as the typical disinfection curve reject (Chick, Eijkman, Arrhenius, etc.) the possibility of explaining it on any frequency basis whatever. They consider that though individual variations may be in part an explanation of departures from the logarithmic type, phenomena of quite a different category are responsible for the logarithmic type itself. Thus Chick in her first paper(3) looks on the bacteria as comparable to molecules with an internal cyclic revolution, and the interaction between poison and organisms as analogous to the inversion of sugar in presence of acid. She later transfers the interaction from the whole bacterium as such to the protein molecules it contains, and expressly rejects the possibility of resistance variations amongst the individuals having any significance when the reaction runs logarithmically. Arrhenius(13) says "there is no doubt that the individual cells in a sample of bacteria or red corpuscles possess a different power

of resistance to deleterious substances," but that the "different lifetime of the different bacteria does not depend in a sensible degree on their different ability to resist the destructive action of the poison."

This objection, however, rests on a misconception or confusion of thought, as has been pointed out by Brooks(11) already. In the frequency

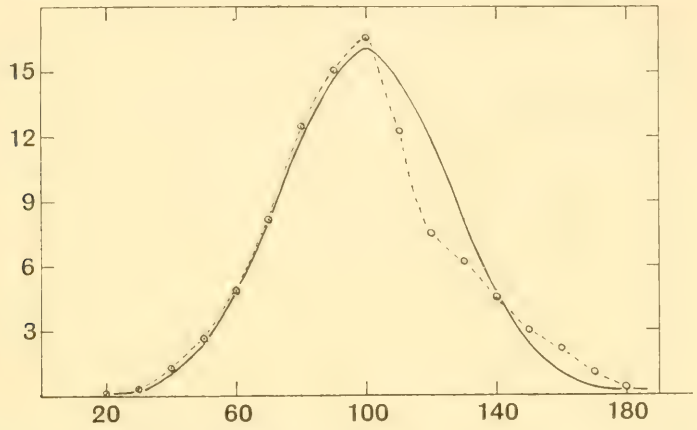

Fig. 8. Frequency curve derived from the 0·4 per cent. phenol sigmoid curve in Fig. 1, together with a normal curve (solid line).

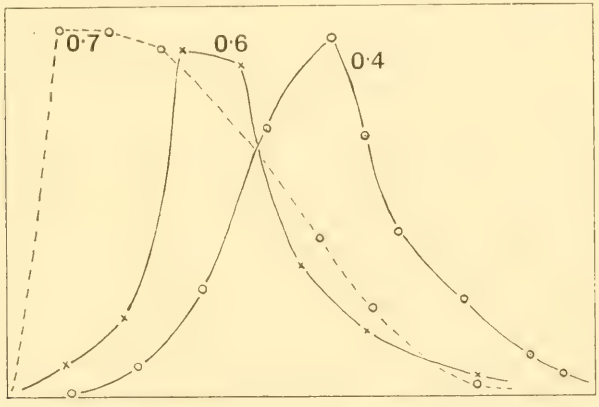

Fig. 9. Frequency curves derived from 0·4, 0·6 and 0·7 per cent. curves, showing increasing departure from normal with increase of phenol.

curve the base line represents resistance, increasing in grade or degree as one proceeds along it. *E.g.* if we were to suppose that the mechanism of resistance is simply the thickness of the spore-envelope, through which the poison has to penetrate, the base-line would represent increasing thickness measured in some selected unit. In the survivor curve our

only measure of resistance is the time taken to kill. But the times taken to kill of the survivor curve will correspond to the grades of resistance of the frequency curve, only if the reaction between spore and poison is proceeding at a uniform rate in all the grades. The two curves will agree only if it takes twice (3, 4 ... n times) as long to overcome 2 (3, 4 ... n) x grades of resistance as it takes to overcome x grades of resistance. They will correspond only if $R/T = k$, where R is resistance and T is time taken to kill. If this be not true, then it is illegitimate to translate the survivor curve directly into a frequency. If the reaction is proceeding faster in some grades than in others, if, *e.g.* it does not take twice as long for the phenol to go through a coat twice the thickness, but a time more or less than twice, then the times of dying do not accurately give the grades of resistance, and the true frequency curve can be derived from the survivor curve only by introducing some factor which will compensate for the change of rate.

This is the fallacy in the argument of those who object that the J-shaped frequency is an impossible form here. They assume tacitly and without evidence that the reaction is proceeding at a uniform rate in all the grades. But it has been shown above that for Botrytis spores at any rate this is not the case. As the strength of phenol is raised, the reaction runs quicker, relatively, in the spores which die early than in those which die late; and it is not a legitimate proceeding to transpose the survivor curve obtained with these high strengths of phenol directly into the frequency curve. What is got by so doing is not the real frequency curve. To get at the real distribution of resistances as they exist in the spores of the suspension it is necessary to introduce a compensating factor which allows for the change of rate.

What general form, applicable to all strengths of phenol, this factor should take, it is not possible at present to determine. The fact that we do get a nearly normal curve from the 0·4 per cent. survivor curve shows that, for that strength of phenol, the reaction is running at a nearly uniform rate in all the grades. If, however, with a sufficiently high strength of phenol, the change in R/T takes some such form as $R/\log T = k$, then we shall get from our original frequency curve a survivor curve that is nearly normal, and from the logarithmic survivor curve a frequency curve approaching normality.

To take an illustration: if we assume that the observed frequency curve shown in Fig. 8 accurately expresses the actual distribution of resistances in the spore suspension (it is not likely to be exactly accurate, because it is not likely that we have exactly hit off the correct strength

of phenol), then when $R/\log T = 50$, we obtain the following table of survivors at the times stated (Table XXI), and the survivor curve shown in Fig. 10. This shows no sigmoid character and approaches the logarithmic type. It is not strictly logarithmic, as is evident from the values of k given in the table; but if we had taken as our basis a true

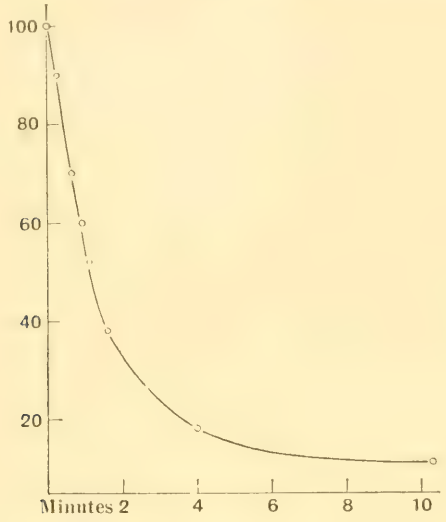

Fig. 10. Survivor curve obtained from the frequency shown in Fig. 8 by allowing for the change of rate with increased phenol.

normal curve, instead of the frequency obtained from the experimental observations, a much better approach to a true logarithmic curve would have been obtained. A strictly logarithmic survivor curve can result only if the frequency curve is slightly skew; but a survivor curve which in experiment closely simulates a logarithmic curve can be got from a strictly normal frequency.

Table XXI.

Time in minutes	Survivors per cent.	k
0	100	—
0·264	90·8	0·158
0·663	70·0	0·233
0·902	60·0	0·245
1·226	50·5	0·241
1·66	38·4	0·250
4·02	18·7	0·172
10·51	8·0	0·104
		Mean 0·200

Again, to take an illustration from anthrax spores as used by Chick: the observed logarithmic curve given by her (Table II, p. 99 in her first paper) is derivable from the frequency shown in Fig. 11 when $R/\log T = 55{\cdot}8$.

It is not suggested that the expression $R/\log T$ is necessarily the correct expression of the relationship between the times required to kill and the resistances. We have not sufficient data to arrive at a correct formulation of this relationship. But it is brought forward to show that by some expression of the kind, which takes into account the alteration in the rate of interaction between cell and poison produced by alteration in the strength of the poison, it is possible to obtain a logarithmic survivor curve from a frequency distribution of approximately normal shape, and that the occurrence of the logarithmic survivor curve does not compel us to look for its explanation in a direction entirely other

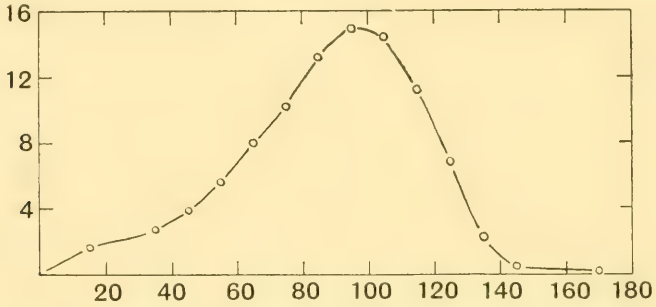

Fig. 11. Transposition of anthrax survivor curve (Chick) into frequency.

than that of differences in degree of resistances. The logarithmic and sigmoid curves are both dependent on the distribution of resistances in the suspension of spores or bacteria. They are both particular cases in the relationship between the resistance and the time required for killing; and may be regarded as different expressions of the same general principle, not as indicating phenomena of entirely different order.

It is not overlooked that this way of regarding the results has its own difficulties, the chief of which is that for a given distribution there is one value, and only one, of $R/\log T$, which gives the best approach to a logarithmic survivor curve. But the value of this expression may vary over a considerable range without any great departure from the logarithmic type, and the value of k in the formula $kt = \log a/a - x$ may also vary correspondingly. It also explains some facts which are otherwise difficult to interpret.

For example, we saw (Tables V, VI) that as we raised the amount of phenol from 0·4 per cent. to 0·7 per cent. the survivor curve tended more and more to assume the logarithmic shape. On reducing the temperature we were able to use 1·0 per cent. phenol, and yet the resulting curve was frankly sigmoid, and even with 1·25 per cent. the sigmoid character is still present. This is intelligible enough, if we consider that the relationship between resistance and killing time will be greatly modified by the lowering of the temperature; but it is difficult to understand if we suppose that the effect of the low temperature is merely to slow the reaction.

It seems clear that we must take into account the change of rate in different grades produced by the change of phenol strength; and it is a considerable simplification if, when this is allowed for, both the sigmoid and logarithmic curves can be derived from the normal or nearly normal distribution of resistances in the spores of the suspension.

I have pleasure in thanking Mr R. A. Fisher for assistance and criticism in the more mathematical aspects of this work.

Summary.

1. It is shown that if Botrytis spores be exposed to the action of 0·4 per cent. phenol, the spores do not all die simultaneously, but some die in a few minutes and some not till two or three hours have elapsed. The curve showing the numbers surviving at different times has a sigmoid shape.

2. If the strength of phenol be progressively raised, the curve becomes less and less sigmoid, approaching the logarithmic type of curve.

3. With the same suspension it is possible to obtain either a logarithmic or a sigmoid curve according to the strength of phenol used.

4. Both types of curve are shown to be explicable on the assumption that the individual spores differ in resistance and that a frequency curve showing the distribution in the resistance grades approaches the normal curve.

5. The influence of the number of spores used is shown to be very considerable; and the consecutive transition from the sigmoid to the logarithmic type occurs, whether we raise the phenol strength, keeping the spore number constant, or reduce the spore number keeping the phenol constant, or use younger and younger spores.

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(Further references on this subject may be found in the papers by Chick and Brooks.)

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BIOLOGICAL STUDIES OF *APHIS RUMICIS* LINN. 1746.[1]

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(With 1 Text-figure.)

Introduction.

In a previous paper (2), the author raised the question as to the influence of the different host plants on the reproductive capacity of *Aphis rumicis* and the resulting varying degree of infestation of different plants. From observations made on certain intermediate hosts of *A. rumicis*, it was found that some species of plants were more heavily infested than others, and an attempt has been made in the following experiments to obtain a numerical expression of the infestation of plants and of the relative susceptibility of the various hosts.

Experiments were carried out during 1913–14 at the Kaiserliche Biologische Anstalt für Land und Forstwirtschaft, Berlin, but owing to the war this series of experiments was terminated in July 1914 and, with the absence of the writer on service, it was not found possible to continue the work until the year 1920.

Some of the 1914 results (series A and B) are now given, together with certain of those for the season 1920 (series C and D).

In the 1914 experiments the aphids used were raised from ova. Ova of *Aphis rumicis* were found on *Euonymus europaeus* near Berlin, on March 25, 1914. These ova hatched out in the laboratory and the larvae of the Fundatrices were reared on *Euonymus* twigs grown under bell jars in wet sand. Some were then transferred to young *Euonymus europaeus* bushes grown in pots. The aphids used throughout the experiments were thus reared in captivity from the ova, through successive viviparous parthenogenetic generations as described below. Thus the

[1] A grant in aid of publication has been received for this communication.

history of the aphids used was known and also the exact generation with which the various plants were infected. Apterous viviparous ♀♀ were used for the infections, as winged forms are often at first restless on the plants, and it is difficult, when dealing with large numbers, to get a fair estimate of reproduction.

The plants were all grown from seed (except *Euonymus*)[1], and were kept covered with muslin bags throughout the experiments, to safeguard against outside infection. The aphids were transferred from one plant to another by means of a fine camel hair brush.

Unfortunately for the 1920 experiments, ova of *Aphis rumicis* could not be found, and the colonies in these experiments were started from an early viviparous parthenogenetic generation found on *Euonymus europaeus* on the laboratory farm on May 10, 1920. This generation was probably the second viviparous generation after the Fundatrix. In future experiments however, it is hoped to breed all the aphids from one egg and one Fundatrix.

It will be seen that the figures obtained in 1920, in general, support the results obtained in 1914.

The plants used in any particular series have as far as practicable been plants of the same age. They were grown in ten-inch pots and kept covered with muslin bags throughout the experiments. Unmanured soil, to which 10 per cent. of sand was added, was used. The plants were kept in a large glass house specially designed with large doors and windows. The great variation in temperature is a source of difficulty, since temperature is an important factor in affecting the development period and the number of aphids produced in a definite period of time. This has to some extent been overcome by arranging as far as possible that infections in each series of experiments were carried out on the same day. The temperature and humidity factors are thus relatively the same for each series of experiments. The temperature (daily, max. and min.) for the period June 25, 1920 to August 4, 1920 in the glass house is shown in Text-fig. 1 below.

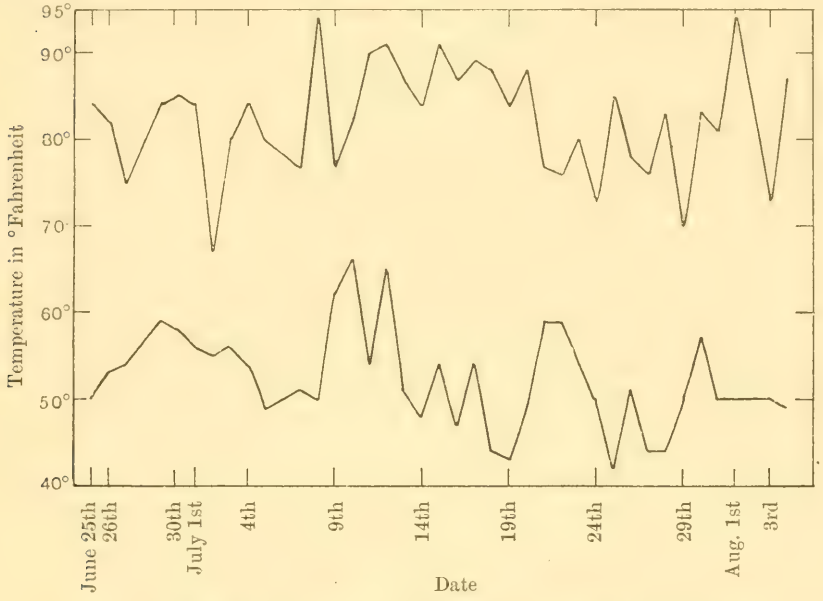
Series A. Infections from *Euonymus* to Different Plants.

(*a*) The aphids were reared throughout on *Euonymus europaeus* from Fundatrices, and the following plants infected with winged migrants of the 3rd v. gen.: broad beans, horse beans, poppies (*Papaver rhoeas*), peas, rumex, dwarf French beans and *Euonymus europaeus*. These plants were "stock" plants for infections to other plants of the same kind.

[1] My thanks are due to Messrs Sutton & Sons who supplied the seeds used in these experiments.

Three plants of each kind mentioned above were infected each with one a. v.[1] ♀, 4th v. gen. from its respective stock plant. After 14 days reproduction, all the aphids produced on each plant were counted.

Below is shown the progress of the infestation on these stock plants and Table I gives the number of aphids produced. The column "winged forms" in all the tables refers only to adults or advanced nymphs recognisable as immature winged forms.



Text-fig. 1. Max. and min. daily temp. of glass house between June 25th and August 4th, 1920.

Broad beans. A. 22. 5. 14. Infected with 9 w. v. ♀♀. 7. 6. 14. 4th v. gen. are all a. v. ♀♀; large size. 18. 6. 14. Many w. v. ♀♀ present; plant heavily infested.

Horse beans. A. 22. 5. 14. Infected with 3 w. v. ♀♀. 7. 6. 14. 4th v. gen. are all a. v. ♀♀. 18. 6. 14. Many w. v. ♀♀ present; plant heavily infested.

Papaver rhoeas. A. 22. 5. 14. Infected with 12 w. v. ♀♀. 9. 6. 14. Reproduction slower than on beans; only a. v. ♀♀ produced. 18. 6. 14. Moderate infestation, several w. v. ♀♀ present. 6. 7. 14. Plants sickly; aphids small size.

Peas. A. 22. 5. 14. Infected with 3 w. v. ♀♀. 9. 6. 14. 4th v. gen. few in numbers but fairly large size, all a. v. ♀♀. 29. 6. 14. Aphids moderate numbers, several w. v. ♀♀ present. 31. 7. 14. Aphids in moderate numbers, many a. v. ♀♀, small size.

Euonymus europaeus. A. 24. 5. 14. Infected with 4 w. v. ♀♀. 10. 6. 14. 4th v. gen. small numbers but fairly large size, all a. v. ♀♀.

[1] a. v. = apterous viviparous; w. v. = winged viviparous.

Rumex. A. 22. 5. 14. Infected with 3 w. v. ♀♀, 3rd v. gen. from *Euonymus*. 8. 6. 14. 4th v. gen. are all a. v. ♀♀; 17. 6. 14. w. v. ♀♀ present, infestation heavy.

Dwarf French beans. A. 22. 5. 14. Infected with 3 w. v. ♀♀. 8. 6. 14. Only 4 aphids alive, the w. v. ♀ mothers dead. 18. 6. 14. All the aphids produced on this plant are dead.

Table I.

Plant	No.	Date adult	Date aphids killed	Aphids produced: Winged forms	Aphids produced: Total
Broad beans	1	6. 6. 14	20. 6. 14	0	1143
,,	2	,,	,,	12	1154
,,	3	,,	,,	2	1192
Horse beans	4	6. 6. 14	20. 6. 14	15	1259
,,	5	,,	,,	13	1239
,,	6	,,	,,	0	902
Papaver rhoeas	7	9. 6. 14	23. 6. 14	5	243
,,	8	,,	,,	9	188
,,	9	,,	,,	16	128
Peas	10	9. 6. 14	23. 6. 14	0	37
,,	11	,,	,,	12	200
,,	12	,,	,,	0	189
Euonymus	13	17. 6. 14	1. 7. 14	2	28
,,	14	,,	,,	0	26
,,	15	Plant destroyed not going well			
Rumex	16	7. 6. 14	21. 6. 14	0	113
,,	17	,,	,,	0	135
,,	18	,,	,,	4	252

(*b*) Three plants of mangolds, red beet and sugar beet were infected, each with one w. v. ♀, 5th gen. from *Euonymus*, and one a. v. ♀, 6th v. gen. was left to reproduce on each plant. The number of aphids on each plant after 14 days are given in Table II.

Table II.

Plant	No.	Date adult	Date aphids killed off	Aphids produced: Winged forms	Aphids produced: Total
Mangolds	1	30. 6. 14	14. 7. 14	0	534
,,	2	,,	,, (Coccinellid larva present)	0	49
,,	3	,,	,,	0	206
Red beet	4	29. 6. 14	13. 7. 14	36	546
,,	5	,,	,,	0	17
,,	6	,,	,,	20	398
Sugar beet	7	29. 6. 14	13. 7. 14	67	622
,,	8	,,	,,	107	445
,,	9	,,	,,	115	696

Series B. Infections from *Rumex* to Different Plants.

(*a*) Fundatrices of *Aphis rumicis* were transferred from *Euonymus* to *Rumex* and the aphids reared.

The following plants (to serve as stock plants) were then infected from *Rumex* with winged migrants of the 3rd v. gen.: broad beans, horse beans, poppies (*Papaver rhoeas*), peas, *Rumex*, and dwarf French beans. From these stock plants three uninfested ones of each of the above species were infected with one a. v. ♀, 4th v. gen., and the number of aphids produced in 14 days recorded. The following notes show the progress of the infestation on the stock plants, and Table III gives the number of aphids produced on the different plants.

Broad beans. R. 25. 5. 14. Infected with 3 w. v. ♀♀. 7. 6. 14. 4th v. gen. are all a. v. ♀♀, some adult, large size. 22. 6. 14. Infestation heavy, aphids small size.

Horse beans. R. 25. 5. 14. Infected with 3 w. v. ♀♀. 2. 6. 14. The w. v. ♀♀ produced nothing, so reinfected with 2 w. v. ♀♀. 11. 6. 14. 4th v. gen. in moderate numbers, large size, all a. v. ♀♀. 22. 6. 14. Infestation heavy, aphids small size.

Peas. R. 2. 6. 14. Infected with 5 w. v. ♀♀. 16. 6. 14. Moderate number of 4th v. gen., all a. v. ♀♀. 15. 7. 14. Many aphids are restless, the plant fairly heavily infested; many w. v. ♀♀ present.

Rumex. R. 24. 5. 14. Infected with 6 w. v. ♀♀. 10. 6. 14. 4th v. gen. all a. v. ♀♀.

Papaver rhoeas. R. 28. 5. 14. Infected with 3 w. v. ♀♀. 2. 6. 14. The w. v. ♀♀ produced nothing, so reinfected with 3 w. v. ♀♀. 15. 6. 14. 4th v. gen. are a. v. ♀♀; not so large as on beans; some adult. 6. 7. 14. Plant heavily infested; many w. v. ♀♀ present; aphids small size.

Table III.

Plant	No.	Date adult	Date killed off	Aphids produced: Winged forms	Aphids produced: Total
Broad beans	1	9. 6. 14	23. 6. 14	2	1465
,,	2	,,	,,	3	1418
,,	3	,,	,,	5	1457
Field beans	4	15. 6. 14	29. 6. 14	0	982
,,	5	,,	,,	8	1223
,,	6	,,	,,	6	1249
Papaver rhoeas	7	15. 6. 14	29. 6. 14	1	151
,,	8	,,	,,	2	82
,,	9	,,	,,	19	216
Peas	10	15. 6. 14	29. 6. 14	0	35
,,	11	,,	,,	4	34
,,	12	,,	,,	0	108
Rumex	13	10. 6. 14	24. 6. 14	0	35
,,	14	,,	,,	1	175
,,	15	,,	,,	0	147

Dwarf French beans not infected, reproduction poor.

Dwarf French beans. R. 25. 5. 14. Infected with 3 w. v. ♀♀. 10. 6. 14. Some aphids 4th v. gen. present, small size, scattered over plant, some dead; w. v. ♀ mothers dead. 15. 6. 14. Only five aphids alive, are a. v. ♀♀, very small size, three adult and producing 5th v. gen. 29. 6. 14. Very few aphids present, small size, dark brown colour.

(*b*) Mangolds, red beet and sugar beet were infected each with one w. v. ♀, 5th v. gen. from *Rumex*. Three plants of each kind were infected, and one a. v. ♀, 6th v. gen., offspring of the w. v. ♀ mother, was left to reproduce on each plant. The total number of aphids produced in 14 days was recorded as shown in Table IV.

Table IV.

Plant	No.	Date adult	Date killed off	Aphids produced: Winged forms	Aphids produced: Total
Mangolds	1	8. 7. 14	22. 7. 14	0	210
,,	2	,,	,,	0	87
,,	3	,,	,,	0	133
Red beet	4	6. 7. 14	20. 7. 14	0	143
,,	5	,,	,,	0	114
,,	6	,,	,,	0	202
Sugar beet	7	6. 7. 14	20. 7. 14	0	82
,,	8	,,	,,	0	238
,,	9	,,	,,	0	146

Series C. Reproduction of *Aphis rumicis* on ten varieties of Broad Beans.

The experiments of 1914 were continued in 1920, by series C and D.

As different varieties of broad beans might give a varying degree of susceptibility, one plant of each of ten such varieties was infected with an a. v. ♀ (offspring of winged migrant from the winter host) to form the stock plants for infecting others. All the seeds were sown on 3. 5. 20. Five plants of each variety were then infected with one a. v. ♀ (offspring of the apterous mother on the stock plant) from the respective stock plant. The apterous mother on each plant began producing on the 25. 6. 20, and the number of aphids after 14 days was counted, the total numbers being given in Table V.

The reproduction over 14 days on a further series of broad beans (Sutton's Prolific Longpod) was tested. The seed was sown on 26. 6. 2. The a. v. ♀ (taken from stock plant No. 6) on each plant, began to reproduce on 20. 7. 20. The results obtained are shown in Table VI.

Table V.

Plant	No.	Aphids produced: Winged	Total
Sutton's Prolific Longpod	1	0	986
,,	2	0	937
,,	3	0	934
,,	4	0	844
,,	5	0	782
,,	6	stock plant	
Sutton's Improved Windsor	7	0	1230
,,	8	0	1016
,,	9	0	978
,,	10	0	937
,,	11	0	925
,,	12	stock plant	
Sutton's Early Longpod	13	0	1021
,,	14	0	882
,,	15	3	783
,,	16	4	567
,,	17	plant destroyed	
,,	18	stock plant	
Johnson's Wonderful	19	0	1028
,,	20	0	970
,,	21	0	910
,,	22	0	795
,,	23	Syrphid larva present	383
,,	24	stock plant	
Taylor's Windsor	25	0	974
,,	26	0	901
,,	27	7	876
,,	28	0	716
,,	29	0	676
,,	30	stock plant	
Sutton's Giant Windsor	31	0	1046
,,	32	0	998
,,	33	0	964
,,	34	3	654
,,	35	16	628
,,	36	stock plant	
Sutton's Green Giant	37	0	951
,,	38	4	865
,,	39	2	745
,,	40	0	733
,,	41	21	591
,,	42	stock plant	
Sutton's Early Mazagan	43	0	1269
,,	44	5	1207
,,	45	0	1106
,,	46	0	987
,,	47	0	930
,,	48	stock plant	
Dwarf Royal Cluster	49	4	947
,,	50	5	857
,,	51	10	720
,,	52	3	698
,,	53	2	510
,,	54	stock plant	
Beck's Dwarf Green Gem	55	2	1290
,,	56	3	977
,,	57	3	953
,,	58	3	781
,,	59	mother died early	90
,,	60	stock plant	

Table VI.

Plant	No.	Aphids produced: Winged	Total
Sutton's Prolific Longpod	1	0	1010
,,	2	0	886
,,	3	0	833
,,	4	3	629
,,	5	0	552
Sutton's Prolific Longpod	6	0	537
,,	7	2	515
,,	8	0	490
,,	9	5	361
,,	10	0	47

Series D. Reproduction of *Aphis rumicis* on Mangolds, Sugar Beet, etc.

One pot each of mangolds, sugar beet, red beet, poppies, peas and dwarf French beans (seed sown 3. 5. 20) was infected with two a. v. ♀♀ from broad beans on 9. 6. 20. These were the stock plants from which four uninfested ones of each species (seed sown 3. 5. 20) were infected with one a. v. ♀. The number of aphids produced in 14 days was recorded, and the reproduction figure for each plant is shown in Table VII. The progress of the infestation of the stock plants is given below.

Table VII.

Plant	No.	Date adult	Date killed off	Aphids produced: Winged	Aphids produced: Total
Sugar beet, Klein Wanzleben	1	29. 6. 20	13. 7. 20	0	156
,, ,,	2	29. 6. 20	13. 7. 20	0	141
,, ,,	3	29. 6. 20	13. 7. 20	5	130
,, ,,	4	29. 6. 20	13. 7. 20	3	66
Sugar beet, Carter's No. 1	5	3. 7. 20	17. 7. 20	0	294
,, ,,	6	1. 7. 20	15. 7. 20	0	119
,, ,,	7	3. 7. 20	17. 7. 20	0	73
,, ,,	8	1. 7. 20	15. 7. 20	0	58
Red beet	9	3. 7. 20	17. 7. 20	0	197
,,	10	2. 7. 20	16. 7. 20	0	164
,,	11	3. 7. 20	17. 7. 20	5	85
,,	12	2. 7. 20	16. 7. 20	10	16
Mangolds	13	1. 7. 20	15. 7. 20	0	201
,,	14	30. 6. 20	14. 7. 20	3	177
,,	15	30. 6. 20	14. 7. 20	10	155
,,	16	30. 6. 20	14. 7. 20	0	42
Poppies, Shirley	17	10. 7. 20	24. 7. 20	4	193
,,	18	7. 7. 20	21. 7. 20	2	178
,,	19	9. 7. 20	23. 7. 20	25	161
,,	20	8. 7. 20	22. 7. 20	33	123
Euonymus	21	1. 7. 20	15. 7. 20	16	117
,,	22	30. 6. 20	14. 7. 20	16	97
,,	23	30. 6. 20	14. 7. 20	0	20
,,	24	30. 6. 20		Aphid mother died	

Mangolds. E. (Carter's Red Chief.) 19. 6. 20. Infected with 2 a. v. ♀♀ from broad beans (offspring of winged migrants on beans). 30. 6. 20. A few aphids produced (three w. v. ♀♀, remainder a. v. ♀♀).

Sugar beet. E. (Carter's No. 1 and Klein Wanzleben.) 19. 6. 20. Infected as above. 29. 6. 20. Two w. v. ♀♀, remainder a. v. ♀♀, some adult and producing next generation.

Red beet. E. (Carter's Perfection.) 19. 6. 20. Infected as above. 30. 6. 20. A few aphids produced (one w. v. ♀, remainder a. v. ♀♀, some adult).

Poppies. E. (Shirley.) 19. 6. 20. Infected as above. 29. 6. 20. Aphids going poorly, small size, plants poor. 5. 7. 20. Aphids now going well, many immature winged forms.

Peas. E. (Carter's Laxtonian Dwarf Pea.) 19. 6. 20. Infected as above. 25. 6. 20. A small colony going; killed off the apterous mothers. 30. 6. 20. Twenty aphids present; small size; three a. v. ♀♀, remainder w. v. ♀♀ (not adult). 14. 7. 20. Both the a. v. ♀♀ and w. v. ♀♀ have produced young. 29. 7. 20. Aphids along mid ribs of leaves, developing slowly, small size. 2. 8. 20. Killed off the aphids, 5 a. v. ♀♀, many immature w. v. ♀♀.

Dwarf French beans. E. (Carter's Canadian Wonder.) 19. 6. 20. Infected as above. 2. 7. 20. Twenty aphids present, scattered over the plant, small size, several dead, one a. v. ♀ adult. 5. 7. 20. Three a. v. ♀♀ adult, very small size, producing young. 26. 7. 20. Aphids increasing in numbers, very small size, all a. v. ♀♀. 28. 7. 20. Plant in flower, aphids on flower heads and young seed pods, slightly larger size. 2. 8. 20. Killed off the aphids; small size; all apterous forms.

Euonymus. E. 16. 6. 20. Infected as above. 29. 6. 20. Moderate number of aphids produced, a. v. ♀♀ and w. v. ♀♀. 20. 7. 20. Many w. v. ♀♀.

General Discussion.

(*a*) *Influence of Food Plants on Reproduction.*

It will be seen from the foregoing data that, given a favourable food plant, and favourable conditions of temperature and humidity, rapid reproduction will result. A comparatively high reproduction figure with rapid development was maintained on broad beans, while on peas, mangolds, sugar beet, red beet and poppies, the figures were considerably lower and the progress of the infestation was somewhat slower.

On dwarf French beans in 1914 the aphids fared very badly, only a few young were produced and the colony eventually died out. Again in 1920 on the variety Carter's Canadian Wonder, the aphids progressed very slowly and poorly, many died and those which remained alive developed into small, dwarf individuals, producing only a few young. However, it was possible to keep them going on the plant from 19. 6. 20 to 2. 8. 20, at which date the plant was flowering, some of the aphids on the young developing pods making a little more progress. The experiment was stopped on 2. 8. 20.

Similarly on peas in 1914 the reproduction figure was low and the development period retarded, although, after two months, a number of aphids were found.

In the 1920 experiments, on dwarf peas (Carter's Laxtonian Pea and Carter's Little Marvel), a few aphids of small size were produced and the development period was prolonged.

Colonies of *Aphis rumicis* have been recorded on scarlet runners (Davidson (2)), on climbing haricot beans (Malanquin and Moitié (6)) and many other plants, where the infestation is not heavy as compared with beans. There is a wide distribution of the species on different kinds of plants which are less or more favourable as indicated by the degree of infestation.

The food of aphids is the sap derived from certain cells of the host plant. Sections of plant tissues fixed with aphids *in situ*, show that the phloem of the vascular bundles is particularly sought after, though cells of the cortex, parenchyma and mesophyll of the leaf, are also tapped by the stylets. This question is being further studied, but it is evident that while the phloem is very important other cells of the plant must be regarded as a source of nourishment.

The constitution of the cell sap may differ considerably in different species, and the hydrogen-ion concentration of cell sap should be investigated in relation to the relative susceptibility of plants to aphid attacks. In this respect Comes' (1) view that the degree of acidity of the cell sap of American vines is in direct relation to the resistance to Phylloxera is important, as is also the recent work on the hydrogen-ion concentration of plant juices and the factors affecting it.

Since physiological characters of plants may be specific and follow Mendelian laws of heredity, this line of investigation, with the possibility of breeding resistant strains, would seem to be a profitable one, and research as to the relative intensity of reproduction on different varieties of the same species is being continued.

Since the chemical composition, or physiological characteristics of the cell sap, is an important factor, one is inclined to the view that the various species of aphids, at any rate in the viviparous parthenogenetic generations, have adapted themselves to many different plants.

In the wide distribution of winged migrants, and the fortuitous adventures of these individuals, they may alight on many different plants—especially in the case of a marked polyphagous species like *Aphis rumicis*—and produce young. Certain plants (more susceptible) provide the necessary stimulus for rapid reproduction. On other plants on the contrary only a moderate reproduction occurs, and on others the colonies may soon die out.

The adaptability of the aphis to its food plants is an important consideration, and is doubtless influenced by the nature of the food plants available. This is a local consideration and probably accounts for the widely different food plants (cultivated or otherwise) to which species

of aphids have adapted themselves in different countries; this being an expression of local races and local conditions. For instance, although my figures of reproduction on sugar beet are comparatively low, it is feasible to expect that, in districts where sugar beet is the most widely distributed favourable food plant, this reproduction figure may be higher. In this respect the reproduction figures on sugar beet for 1914 and 1920 are interesting. To quote an example; Davis (1909) has shown that *Aphis maidis* Fitch, prefers broom corn to Indian corn or sorghum. In the struggle for existence against adverse conditions, the chances of survival are greater for the strain of aphis produced on plants which cause a high reproduction figure.

It seems probable, although the data obtained from my experiments are not sufficient to draw conclusions, that reproduction on certain intermediate hosts may be affected by the nature of the previous host on which the aphids were reared. In Tables II and IV it will be seen that mangolds, red beet and sugar beet, infected with a strain of *Aphis rumicis* which had been reared for several generations on *Euonymus europaeus*, gave a higher reproduction figure than when infected with a strain reared on *Rumex*. Similarly, higher figures were obtained on these plants when they were infected with strains of *Aphis rumicis* reared on beans and poppies. This question, however, requires further investigation with reference to the factors concerned, especially the condition of the plant and temperature conditions.

There are the further questions of the influence of climate, varying soil conditions, agricultural methods, and the manurial treatment of crops, on the constitution of the cell sap of plants. The importance of manurial treatment in relation to the susceptibility of varieties of wheat to attacks of rust fungus is well known. According to Comes, nitrogenous fertilizers stimulate the cells of the plants, causing the tissues to be more juicy and diminishing the acidity of the cell sap.

The ten varieties of broad beans experimented with (Table V) showed little difference in the degree of susceptibility to attacks of *Aphis rumicis*. One might infer that the varieties Sutton's Improved Windsor and Early Mazagan are slightly more susceptible than the other varieties, and that Dwarf Royal Cluster, Sutton's Green Giant and Taylor's Windsor are slightly less susceptible. It is clear however that these strains of beans are too closely allied to give any striking difference. In any case, they only represent a very limited number of the varieties obtainable. Experiments are being continued with varieties of field beans.

The wide range of variation in the numbers on each variety, is probably largely due to the variable fertility of the agamic females.

In August and September 1913, 30 a. v. ♀♀, offspring of wild w. v. ♀♀ of *Aphis rumicis*, were isolated on broad bean in a greenhouse, with a temperature varying from 62° F. to 85° F. The daily reproduction and total offspring produced by each mother were recorded. The maximum number of young born in any one day was 8. The minimum being 1. The maximum total number produced was 45 and the minimum 6. Three examples are given showing a low figure, a medium figure and a maximum figure of reproduction.

Temp.° F.	62	67	77	70	77	77	85	80	70	77	65	70	65	Average daily production	Total aphids produced
August, 1913	19	20	21	22	23	24	25	26	27	28	29	30	31		
Plant 1	0	0	3	1	2	1	0	3	2	0	0	0	—	1·7	12
,, 2	6	2	2	6	0	1	0	1	0	2	0	0	—	2·0	20
,, 3	5	5	2	5	2	5	3	4	4	4	6	0	—	4·1	45

It should be noted, however, that these aphids were taken towards the end of the season, and the history of the previous generation was not known.

There would appear to be some relation between the time of the year and the degree of infestation of plants.

Recorded observations of marked decrease in the reproduction of aphids during high summer temperatures, indicate an association of temperature and the season of the year with the "dryness" of food plants as contrasted with the young succulent growth of spring and early summer. It seems feasible to expect as a result of these factors, a seasonal adaptation of aphids to the varying seasonal conditions. The number of the viviparous generation used in experiments may therefore be an important consideration when results obtained at different periods of the season are compared.

In my experiments, there was a marked decrease in the numbers of aphids produced towards September and October, probably to some extent due to lower temperature conditions and to the appearance of sexual forms in the cultures; but cultures, carried on throughout winter in a warm greenhouse, also produced few aphids in comparison with the reproduction in June. To some extent the appearance of sexual forms in each generation accounts for this, but my observations indicate that the greatest reproduction occurs with the early viviparous generations.

Experience in breeding aphids shows that in investigating the reproduction on different hosts, the following factors must be considered:

(*a*) The physiological condition of the plant, especially with regard to its age, temperature, light and food.
(*b*) Temperature and humidity conditions.
(*c*) The history of the previous generations, the generation of the aphids used, and the kind of plants on which they have been reared.
(*d*) Whether winged or apterous agamic females are used.
(*e*) The variability in reproductive power of the individual agamic females.

(*b*) *Influence of Food Plants on the Characters of the Species.*

The quantity and quality of the food are very important factors in affecting the appearance of *Aphis rumicis*, especially in regard to colour and size. When kept for several generations on *Euonymus* the aphids in the later generations are very much smaller than the earlier generations, but by cutting back young trees, thus producing a succession of young growth, the aphids were carried on the spindle tree for several months. They were somewhat smaller than the earlier generations, and considerably smaller than the corresponding generations on broad beans, also the reproductive capacity on *Euonymus* was greatly inferior. On broad beans the aphids were always large, healthy individuals, except in the case of very heavily infested plants. On poppies they were somewhat smaller. On mangolds, sugar beet, and red beet they were also smaller than on beans. On dwarf peas they were quite small individuals but healthy in appearance, while on dwarf French beans they were extremely small, dwarf individuals with very low producing power.

There is in a general way some correlation between the size of the individuals on the host plant, and the number of aphids produced. This correlation between size and fertility is also indicated in Warren's(7) statistical examination of *Hyalopterous trirhoda* Walker.

Ewing(3) in his interesting statistical study of *Aphis avenae* Fab. has shown the great range of fluctuating variations over a number of parthenogenetic generations, these variations being individual and not inherited. Extremely high and low variations were traced to the effects of food and temperature conditions. Temperature will of course also indirectly affect the food factor, in so far as it influences the metabolism of the plants.

With *Aphis rumicis* it was found that small agamic females from dwarf French beans, when transferred to beans, produced young which developed into normal size.

(c) *The Influence of Temperature and Humidity on the Development of the Species.*

It was observed throughout the experiments that low temperatures retarded the development period and the production of young over a given period. For this reason figures of reproduction for one period cannot be safely compared with those for another period, unless due regard is paid to temperature conditions. Further it appears probable that the maximum reproduction occurring in early summer, with a decreasing reproduction later in the season, may be an expression of adaptation, and that even with favourable conditions of food and temperature with later generations, later in the year, the high reproduction figures of earlier generations are not obtained. It is interesting to compare Tables V and VI in this respect. Recorded observations show that there is a decrease in the number of aphids during high summer temperatures and experiment demonstrates that abnormally high temperatures restrict the development: it has been pointed out above, that the association of high summer temperatures with the "dryness" of the food plants at that period, involves the factor of food conditions with a possibility of a seasonal adaptation of aphids.

Aphis rumicis could not be reared in winter in the warm dry air of the laboratory, most of the young dying before reaching maturity; whereas in a similar temperature in the humid atmosphere of the greenhouse they developed normally.

Headlee (4), found with *Toxoptera graminum* Rondani, that with a constant humidity of 75 per cent., the development period from birth to maturity varied according to the temperature. Variations in atmospheric humidity (between about 37 per cent. to 100 per cent.) did not have any marked effect on the metabolism of the aphids. Similarly variations of 20 degrees or so about the optimum temperature did not produce any marked effect.

Klodnitski (5) also showed the retarding effect of low temperatures on other species of aphids. It would appear that there is an optimum temperature for development which may vary for different species.

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SOME RELATIONSHIPS OF ECONOMIC BIOLOGY[1]

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BEING THE PRESIDENTIAL ADDRESS DELIVERED TO THE ASSOCIATION OF ECONOMIC BIOLOGISTS AT THE ANNUAL GENERAL MEETING, FEBRUARY 18, 1921

I have to thank you for the opportunity of presiding over our deliberations during the past year. Your consideration and the kindness of my colleagues on Council have made the privilege a pleasure. That pleasure would have remained unmixed but for a reminder that to-day is the occasion for an annual address.

Our Secretary in making this intimation has kindly added that although such addresses usually deal with the wider issues of biology there is no established precedent which need be followed. Grateful for this assurance I will restrict my remarks to some outline of the varying relationships which our work as economic biologists has borne to the activities by which natural knowledge is gained.

It is convenient to consider separately the philosophical relationship between economic and natural study, and the development of that practical relationship familiar to those whose personal recollections go a generation back.

As economic work antedates philosophical study it would seem proper to consider the practical relationship first. But it is more convenient to reverse this order. In this country one philosophical relationship between economic and natural study was clearly defined by the middle of the seventeenth century, and the evolution of that conception helps us to understand why effect was not given to it till our own day.

Our Association is concerned with Economic Biology. Economic Biology is conditioned as other natural studies are. Its business is to deal with natural things. These assort themselves into two groups. Regarding most things all we can say is that they exist and that observation and experiment have revealed some of their effects. Only

[1] A grant in aid of publication has been received for this communication.

as regards a few may we venture to imagine that we understand their causes or know their nature.

The economic worker shares with the seeker after truth for truth's sake the hope of detecting natural effects hitherto unsuspected. He may even aspire to increasing the number of known natural things. If his duties debar him from attempts to explain natural causes, they at least afford him reason for wishing that his knowledge were less circumscribed than it is.

The existence in divers degrees of these hopes and wishes led to the old subdivision of students of nature into those who are "learned" and those who are "curious." Both rely on the aid of the same two studies: History, which records what is known; and Philosophy, which considers what may be knowable. Both depend for success on the same two faculties: Observation which, where fully trained, occasionally perceives what is obvious; and Imagination which, when properly controlled, sometimes suggests how knowledge may be sought.

The "learned" are largely incited to study by the effects of natural qualities. They keep imagination under control. Their progress both in conception and in accomplishment is *per gradum*. The "curious" are more attracted towards enquiry into natural causes. They use imagination to devise theories. If the conception which gives rise to a hypothesis and to the experiment which tests it be philosophical, the progress, if any, which they make is *per saltum*. Whatever may be the case in other studies Economic Biology is beholden to both.

The peculiar merits of each sometimes develop into defects. If the "learned" fear to employ imagination their progress is slight. If they rely implicitly on authority their progress may cease. If the "curious" become the victims of imagination they may lose ground by accepting explanations of natural causes which neither observation nor experiment can confirm.

Philosophical study has been most impressed by these defects. The term "learned" has been relegated to literature; the term "curious" has fallen out of use. The pejorative significance which these names have acquired among students of nature has its disadvantages. The economic biologist has reason to know that the discontinuance of this classification has neither altered the conditions that led to its adoption, nor modified the circumstances that dictated its abandonment. He is, however, left to discover, after he has embarked on economic study, how valid and vital the old distinction was.

Yet academic natural study was perhaps justified in discarding these

terms. She thus disavowed disciples wedded to authority or satisfied with conjecture. She encouraged philosophical observation and experiment by abandoning systems of nature founded on unconfirmed statements and natural laws based on unverified hypotheses. She placed natural enquiry on a sounder basis. She realised that the promotion of natural knowledge is a means to that higher end, its application for use or for further discovery. As natural things were put to practical use before the study of natural effects was undertaken, the acceptance of the novel philosophical principle that the application of natural knowledge is a human duty, was at the same time a recognition of an established historical fact.

When the habit of noticing natural effects began there was little tendency to enquire into natural causes. This was not because the latter were overlooked; it was owing to a belief that natural causes are explicable by the principles of sympathetic magic which are assumed though never defined by savage races and are still at times subconsciously accepted by races that claim to be refined.

A change takes place when the perception of natural effects is supplemented by spiritual conceptions. Revelation, which faith accepts, is antithetic to the principles of sympathetic magic. It undertakes to do more than these principles can. Sympathetic magic only explains the causes, revelation professes to explain the effects of natural qualities as well. The explanation is less precise. But it is more comprehensive and effectively inhibits natural curiosity. The only human interest which revelation does not always destroy is that in the "virtues" of things.

Some faiths have been helped by specialisation in this interest. Their leaders have found it profitable to cultivate an expert acquaintance with natural effects. The reflected spiritual quality thus imparted to them has at times prolonged sacerdotal authority over the vulgar when profane study has left the priest relatively imperfectly informed.

Most spiritual laws find their fulfilment in new faiths. A fresh spiritual impulse as a rule succeeds more rapidly if its pioneers possess miraculous powers. Where these powers are extensive, the new faith regards the application and the promotion of natural knowledge as equally uncalled for, and the applied worker finds his occupation gone.

Grace, however, may diminish as influence grows and in one familiar case, where spiritual authority eventually brought temporal power under control, the grace failed to protect the State against external aggression. This accident modified spiritual attitude towards natural knowledge. Promotion of natural knowledge remained an offence; the use of natural

knowledge was re-sanctioned and the applied worker once more came into his own.

We may regret the restriction. We have, however, to admit that the treatment accorded to natural study and spiritual discussion was strictly impartial. Dogma became the substitute for both and remained in operation for a millennium.

When effective objection to dogma eventually arose, the protest against the spiritual variety found greatest popular support. But the less articulate revolt against natural dogma was that whose effects have been most enduring. In some countries the desire for spiritual emancipation was slight. There ecclesiastical authority encouraged natural enquiry, provided the results published did not controvert scripture. In lands where a reformed spiritual doctrine became dominant the tendency was to make the embargo on natural study more stringent. Protestant conviction emulated primitive faith in its condemnation of profane efforts to apply natural knowledge for the good of the community.

Few of those Governments that with the help of popular antagonism to spiritual dogma had thrown off one ecclesiastical yoke were minded to subject themselves to another. They therefore encouraged the promotion of natural knowledge on the understanding that any new knowledge secured be improved in the public interest.

The application of natural knowledge thus encouraged by the State and assented to by philosophy, had as its basis the promotion of such knowledge. The promotion of natural knowledge involves two operations: the search for further truth and the co-ordination of new facts with pre-existing knowledge. The search for truth is a constructive activity involving original enquiry. The co-ordination of knowledge is a critical activity which may reveal the need for research into subjects hitherto accepted as explained. Enquiry and research are only preliminaries to consideration as to how far and in what way their results may be improved. In applying such results it was agreed that improvement for use take precedence over improvement for discovery.

Application of knowledge for use takes two forms. The knowledge may be employed to promote intellectual development or to supply material needs. The former usage gave rise to studies once known as "moral history" and "moral philosophy," the latter to studies termed "natural history" and "natural philosophy." The first of these terms has disappeared; the others are still familiar, though in all the significance of the term has changed.

Economic biologists have to read old books as well as new. They therefore know that early naturalists wrote "Moral and Natural Histories," dealing with "everything performed by man or produced by nature" in particular regions. This union by the field-worker of subjects which the scholar can keep apart is due to the community of interest of "moral" and "natural" study in the "qualities" of things. The "moral historian" deals primarily with the "uses" dependent on the existence of definite "qualities" whose presence compels the "natural historian" to detail the "characters" of the things in which they are manifested. The "economic naturalist" must attend both to the "characters" and the "virtues" of things, the latter being the term employed to include both "qualities" and "uses."

Economic study is thus something complete in itself, which also links "moral" and "natural" history. Hence the evolution of that alternative scheme of knowledge wherein the various philosophical and historical studies were aggregated as "sciences," while the activities concerned in the application of knowledge were subdivided in terms of the underlying purpose. The agencies charged with the application of knowledge in doctrine were designated "letters"; the technical pursuits involving the application of knowledge in practice were styled "arts." In this brotherhood of the "Sciences, Letters and Arts," whose foundation was contemporary with the evolution of the philosophical scheme for the "improvement of natural knowledge," "application" was collateral with instead of consequent upon "promotion" of knowledge.

"Natural history" and "natural philosophy" at first bestowed appropriate attention upon the "characters" and "virtues" of all natural things. Soon, however, both felt the influence of the distinction between things organic and things inorganic. By degrees "natural philosophy" concentrated her attention on the inorganic, leaving the organic to be dealt with elsewhere. This habit led "natural philosophy," by an easy transition, to the study of physical and chemical problems, leaving those problems connected with organic structure and function to be dealt with by the correlated economic agencies. When "natural philosophy" took cognisance of physiological problems at all she did so because these problems, though of biological origin, were "statical" in character. This practice reacted on "natural history" whose duty is to codify and co-ordinate natural knowledge and to co-operate with "natural philosophy" in applying it. The understanding tacitly reached was that "natural history" should advance as well as co-ordinate knowledge regarding the characters and virtues of organisms, while "natural philo-

sophy" undertook to co-ordinate as well as to advance physical and chemical truth. A subdivision of natural study according to subject thus replaced one according to method.

This change was convenient. Its only disadvantage was the retention of a terminology no longer appropriate. History is of equal consequence to physics and to biology; co-ordination of knowledge is as essential to inorganic as to organic study. Biology makes use of philosophy just as physics and chemistry do. If philosophy enabled physics to establish the principle of "conservation of energy," she helped what we still term "natural history" to formulate the doctrine of "evolution."

The new arrangement was already recognised before the end of the first quarter of the eighteenth century and "natural history," as then understood, has observed her obligation ever since. The effect of the change on economic biology is what interests us now. Our study obtained from "natural history" that philosophical acquaintance with the characters and virtues of animals and plants as organisms which underlies "domestication." For information regarding the structure and functions of the animal and the plant as mechanisms, economic biology was indebted to the Institutes of Medicine, already a powerful technical study, and the Institutes of Rural Economy, one still relatively backward.

By the middle of the eighteenth century the mechanical arts decided to follow the example of medicine. Aware of the progress due to technical studies evolved by the art of healing, "natural philosophy" approved the adoption by the engineer of technical in place of philosophical methods in securing the knowledge he required. This enabled "natural philosophy" to limit her activities to the promotion of inorganic natural knowledge. Relief from the task of applying knowledge induced an increase in the activity involved in codification. This new impulse spread from inorganic to organic philosophical study and natural knowledge underwent an "encyclopaedic" phase. The more intensive interest in the characters and virtues of animals and plants thus induced was not accompanied by, and is thought by some to have inhibited, a comparable activity in the study of organic structure and function. During this period therefore the relationship of economic biology to natural study was hardly affected.

Among the tasks undertaken by physical and chemical technique at the instance of the mechanical arts were those of providing biological study with instruments more effective and methods more precise. The success which attended these efforts was such that within the first

generation of the nineteenth century, philosophical enquiry into animal-structure and plant-structure enabled "natural history" to co-ordinate knowledge and "economic study" to apply it with an assurance hitherto impossible. The technical study of function on the nutritive side had taught medicine that health and disease are opposite faces of one shield, and had satisfied husbandry that plant-growth is more than a mechanical process. Pathological technique now told the physician how to diagnose; chemical technique felt justified in teaching the farmer how to till. Such techniques received academic recognition and were raised to the status of studies comparable in importance with "natural history," which now underwent fission into branches restricted respectively to animals and vegetables. But while in both cases the study of organic structure could now be linked with the earlier interest in the characters and virtues of living things, neither branch of "natural history" undertook the philosophical study of organic function.

The philosophical study of reproduction had not yet set in; the technical study of this subject, which played so important a part in the early history of mankind, had degenerated into a traditional empiricism. The technical study of nutritive function was shunned by both branches of "natural history" for two rather different reasons. The study of animal nutrition was the prescriptive right of medicine; the study of plant nutrition from the standpoint of the substratum was so ardently pursued by chemistry that the part played by the plant itself hardly interested husbandry.

The academic incorporation of techniques older than their correlated philosophical studies afforded evidence of a deeper change. The seventeenth century interest in the improvement of natural knowledge had been narrowed in the eighteenth through the delegation to technical study of the duty of applying such knowledge. The eighteenth century interest in the promotion of natural knowledge had been further narrowed in the nineteenth by the exaggeration of the distaste for "encyclopaedism" into an aversion towards systematic work. Academic interest was concentrated upon the philosophical increase of natural knowledge and its application in doctrine. These two activities were now amalgamated in the comprehensive term "natural science."

Every old technique has been the product of some correlated art, at whose service the activities of the technique were placed. The co-ordination and application of the knowledge gained were duties appertaining to the art concerned. Yet every sound technique eventually reaches a stage when it seeks new knowledge on its own initiative. The

correlated art still codifies and employs the knowledge thus supplied. But the technique now puts this new knowledge to doctrinal use by instructing the art it serves.

Technical and philosophical studies thus move in opposite directions until a point is reached at which they enjoy community of interest. Alliance is inevitable. Equally natural is the adoption of the view, characteristic of the latter half of the nineteenth century, that the "advancement of science" is the only activity which deserves academic distinction.

Academic distinction, however, even at the close of last century, was not the only incentive to philosophical study. Some there were who regarded the search for truth as its own reward. With others the public good was a sufficient stimulus to effort. Enquiry into the effects of things organic, which chiefly concerns us, was thus, a generation ago, undertaken by three distinct agencies: Natural History, directed to the promotion of knowledge by increasing and co-ordinating it, as a preliminary to considering how best it may be improved; Academic Science, engaged in the advancement of natural knowledge, with a view to its employment for discovery and doctrine; and Applied Biology, occupied in seeking new and sifting old knowledge, with the object of furthering economic ends.

Economic Biology endeavours to serve the various human arts as their own old technical studies did. But that service is now rendered *solute nec abjecte*, and the new technologies devised to carry it out derive their methods from Academic Science and their inspiration from Natural History.

There need be no antagonism between agencies whose outlooks differ. Any misunderstandings that may occur find their explanation in a pardonable inability to appreciate unfamiliar points of view. The natural historian must regard his facts from the standpoint of their bearing on the duties of his study to natural knowledge as a whole; the academic enquirer may concentrate his attention on the new facts he secures; the economic biologist must regard his facts from the standpoint of their value to the industrial interests he desires to assist.

The great impediment to cordial intercourse between Academic Science and Natural History during the closing generation of last century was the obstinacy with which the latter agency still pursued the always difficult and often thankless tasks of determination and classification. The Natural History of Animals was allowed to retain academic status under the style and title of "Comparative Anatomy,"

on the understanding that philosophical interest in the characters and structure of the subjects of study be discreetly veiled. But the Natural History of Plants was formally debarred from academic intercourse and the philosophical study of nutritive plant-function has been installed in its stead. The sincerity of this objection to co-ordination is best revealed by the inconsistency of academic attitude towards the philosophical study of plant-structure. For a time this study shared, on terms of equality with plant-physiology, the position of which Natural History has been deprived. Unfortunately the facts of plant-structure lead to advances in philosophical classification and the study of plant-structure has become so suspect that it is now the fashion to declare that the truths of vegetable morphology are unprofitable for purposes of doctrine. But while Academic Science has to some extent endorsed this view, the "Comparative Anatomy of Plants" has been tacitly conceded certain academic privileges. The plant-morphologist may still continue in the palaeontological field the philosophical tasks of reconstructing vanished vegetations and determining geological horizons. Academic Science has also made an exception in favour of the morphological technology which endeavours to explain the causes of those nutritive functions whose effects interest the plant-physiologist, and attempts to demonstrate the mechanism of those reproductive functions whose effects extend to the domain of moral study.

Economic Biologists experience no difficulty in appreciating the determinative interest of the Natural Historian. We benefit at every turn from the results of historical study regarding the characters and virtues of organic entities, and are glad of the assistance the naturalist can give as regards the philosophical limitation of the essences with which we deal. The ardour of the Natural Historian in systematic study has placed Economic Biology under further obligation: "Plant Pathology" has been evolved from the natural histories of invertebrate creatures and cryptogamic plants, therein differing from "Animal Pathology" which is a bye-product of Animal Physiology. We have, as Economic Biologists, a stronger bond of sympathy still with the Natural Historian. We have discovered in the course of our own work that in the soil we have a fauna and a flora as complex and as important as those that appear upon it. We realise that our first business is to codify this new knowledge. In accomplishing this task we may find it expedient to adopt methods of our own. But we appreciate already that we may encounter difficulties with which Natural History is familiar, and that we must attack our problem in her spirit.

Economic Biology has reason to know that the interdependence of Natural History and Academic Science is equally marked. Oecology, whose results we apply to everyday affairs, has shown us that diversity of purpose is no barrier to community of interest. Economic Biology realises, even if Academic Science and Natural History do not, that the field-naturalist cannot escape being an Oecologist, and that the Oecologist is a Natural Historian in spite of himself.

Notwithstanding the suspicions she has aroused, Morphology is the special study that, perhaps unwittingly, does most to harmonise the divers activities of Natural History, Economic Biology and Academic Science. Cytology, which regards herself as a self-contained study, is nevertheless a branch of Morphology which plays a part in assisting all three. Natural History relies on Cytology for the philosophical knowledge on which her most fundamental systematic conceptions are based. Vegetable Technology is equally beholden to Cytology for information which enables industrial interests to overcome practical difficulties. Cytology deserves the credit of despising an antiquated opprobrium which Academic Science is prone to dread. No study can be so "learned"; the Cytologist assures us that explanations of natural effects offered by Natural History, Academic Science or Economic Biology can only be accepted when confirmed by cytological methods. No study has been more "curious"; Cytology not only explains what the structures connected with nutritive and reproductive functions do, but ventures to tell us how they act.

A generation ago both history and economy were inclined to regard natural knowledge of the characters and virtues of organised things and natural knowledge of organic structure and function as being different in kind. A corollary was the tendency to narrow the incidence of the term Economic Biology to studies connected with the application of functional and structural knowledge. This tendency has disappeared with the idea that underlay it. Even Academic Science, under the consolidating stress of conflict, so far discarded prepossession as to vie with Natural History and Economic Biology in devoting her energies to the task of applying natural knowledge at first hand.

This happy concurrence of the agencies concerned with the improvement of natural knowledge restored a philosophical conception, evolved two and a half centuries ago and kept alive by Natural History, through good report and ill, ever since. It also restored the practical attitude towards Economic Biology adopted by early man. He was sufficiently interested, from primitive times, in the effects of organic

things and in their nutritive and reproductive functions, to be guided by his knowledge as to what he might use and how he should mate. Poetic faculty led him "to project himself into nature" in search of an explanation of the causes of things. While still a hunter he was guided by experience to devise codes regulating the breeding and selection of his own kind. Later, as a herdsman, he extended his interest to include animal-nutrition and animal-breeding. Later still, as a tiller of the soil, this interest embraced plant-nutrition and plant-selection.

When poetic intuition was replaced by divine inspiration, practical expedient yielded to priestly ordinance. The interest in breeding and selection, cankered at the root by Essene doctrine, found an imperfect outlet in "domestication."

Two hundred years ago the subject of plant-nutrition was taken in hand by philosophy. But the study was referred to "statics" and the opportunity was not improved. A century later, the study was resumed, but its improvement was again postponed in deference to the intelligible respect entertained by husbandry for chemistry. It was not till some half a century ago that the philosophical investigation of plant-nutrition at last came into its kingdom.

The earliest attempt at the philosophical study of reproductive-function was practically contemporaneous with this third happy effort to treat nutritive-function on philosophical lines. But Genetic study was then less fortunate than its cognate and its results were overlooked by Academic Science.

Within our generation that study has been energetically resumed. Imbued throughout with the Natural History spirit, it has placed practical animal-breeding on a philosophical basis and has made economic plant-breeding a possibility. The immediate value of the results to Economic Biology and their potential consequence to Moral Philosophy are already fully understood.

I must apologise for having dwelt at such length on facts that are familiar and relationships that are well appreciated. We Economic Biologists are, we know, but a feeble folk, "hewers of wood and drawers of water" to some of the "sciences" and many of the "arts." But this very circumstance makes us "citizens of no mean city" and it may not be wholly amiss, when taking counsel together, to remind ourselves, sometimes, of the fact.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

February 18th, 1921.

Presidential Address.

Some Relationships of Economic Biology—Sir David Prain.

March 11th, 1921.

EXHIBITS.

I. (*a*) Cultures of *Polyopeus*.

(*b*) Demonstration of the Enzymic Action of *Polyopeus* in Solid Nutrient Media containing Starch—A. S. Horne.

II. (*a*) Some Cultures of the Causal Organism of a Potato Disease.

(*b*) A Novel Method of Inoculation of Potato Tubers—S. G. Paine.

III. The Differentiation of Biological Forms of *Puccinia Graminis Tritici* by the Sorus Characters on Infected Pure Lines of Wheat. (From the Department of Plant Pathology of the University of Minnesota)—Wm. B. Brierley.

IV. Potato Tubers infected simultaneously by Corky Scab and Wart Disease—G. C. Gough.

PAPERS.

I. The Cells of Plant Tissues in Relation to Cell Sap as the Food of Aphids—J. Davidson.

II. Ceylon Ambrosia Beetles and their Relation to Problems of Plant Physiology—E. R. Speyer.

April 22nd, 1921.

PAPERS.

I. Green Plant Matter as a Decoy for *Actinomyces Scabies* in the Soil—W. A. Millard.

II. The Action of Bacteria and Protozoa in conserving the Nitrogen in Sewage—E. H. Richards.

III. On the Methods of Infection of the Apple Canker Fungus—S. R. Wiltshire.

EDITORIAL NOTE

At the General Meeting of the Association of Economic Biologists held in the Imperial College of Science on Feb. 18th the Treasurer reported that the cost of publication of Vol. VII of *The Annals of Applied Biology* had been so great as to make material financial retrenchment a necessity. The Publications Committee therefore put to the meeting the following proposals which were carried unanimously.

(1) Reduction in size of papers.

All papers published in the *Annals* must be condensed to the utmost and for this year no paper will be accepted exceeding 8000 words in length.

(2) Tabular Matter.

As the printing of tables is a costly process all contributors are requested to reduce tabular matter to the absolute minimum, and if possible to arrange the data in line or graphic form.

(3) Plates and Text-Figures.

All plates must be paid for by the contributors and as regards the text-figures a limited number will be allowed by the Publications Committee.

(4) Reduction in Number of Pages.

Fewer papers will be published in Vol. VIII, each part being limited to 50 pages, with discretion to call an individual part a double number. As far as possible each part will contain papers dealing with different aspects of economic biology.

The above restrictions apply only to papers published at the expense of the Association and not necessarily to those papers for which "grants in aid" have been received.

It is hoped that the reductions outlined above will only be operative for the current year since it is anticipated that in 1922 it will be possible to revert to improved conditions.

All contributors are asked to read carefully the instructions on the back cover of the *Annals*.

ON THE SUPPOSED OCCURRENCE OF SEEDLING INFECTION OF WHEAT BY MEANS OF RUSTED GRAINS

BY W. L. WATERHOUSE, B.Sc., AGR.,

Walter and Eliza Hall Research Fellow of the University of Sydney.

(Department of Plant Physiology and Pathology, Imperial College of Science and Technology.)

AN important paper by Hungerford has recently appeared in the *Jour. Ag. Research*, vol. XIX. 15 June, 1920, p. 257, dealing with "Rust in Seed Wheat in Relation to Seedling Infection." As the result of careful and exhaustive experiments he states that, contrary to results reported by a number of previous workers, "stemrust (*Puccinia graminis tritici*, E. and H.) is not transmitted from one wheat crop to the next by means of infected seed grain. Further, in the writer's judgment, the occurrence of stemrust sori in the pericarp of the caryopses of grains and grasses has no especial significance, but the infection spreads to these tissues just as it does from an infection point in any of the vegetative parts of the plant" (p. 275).

The same writer gives a comprehensive summary of past work on this question and a full bibliography.

The present investigation was undertaken to see whether a histological examination of rusted grains during germination and the early stages of growth would throw any light on the problem. The results obtained confirm Hungerford's work and are briefly reported here.

In April, 1920, grain was obtained from a crop of wheat which was grown in Pembrokeshire in 1919 and badly rusted. Stubble left in the field was frequently found black with teleutosori, which were also abundant on awns, chaff and fragments of the rachis found mixed with the grain. The presence of teleutosori at the hilum end of a number of grains was determined and these grains were picked out. Several counts showed the percentage of rusted grain in the sample to vary from about 0·5 to 1·0 per cent. of the whole. The percentage of abnormal grains of a scabby nature was higher than this, and spores of the *Fusarium* and *Helminthosporium* type were obtained in scrapings from some of these.

Of the rust-infected grains, one portion was subjected to the hot-water treatment, the other portion left untreated. As controls, similar portions of grain on which no sori could be detected were used, part being treated and part untreated. All were germinated at a temperature of 15–18° C., and when the shoots were about half an inch long, planted in pots which were kept in a glasshouse. There was no observable difference in the germination of the rusted and healthy grains. At intervals during germination and growth seedlings were removed and portions of them fixed in Flemming's weaker solution, embedded and sectioned. The plants were under observation for a period of three months, during which time no rust appeared.

Sections of the infected grains showed that many of the numerous teleutosori at the hilum end were internal sori. In the germinating grains there was no indication of any development of the rust mycelium underlying the sori; furthermore, it had every appearance of being a dead mycelium as described by Hungerford. In many instances, irrespective of whether the original grain was rusted or healthy, treated or untreated, fungal hyphae were present in the developing seedling. They were generally of an intracellular nature. In one instance a *Fusarium* type of spore was noted in a mass of mycelium growing on the outer layers of the scutellum, and in other cases the hyphae belonged to a species of *Helminthosporium*, spores of this type occurring close to the outer masses of mycelium. Nothing in the nature of the "palmella-like" developments reported by Pritchard (*Phytopathology*, 1911, I. p. 150) was found. Cases were observed in which hyphae were present in the outer layers of the base of the seedling stem, but they were larger than rust hyphae and were intracellular in growth.

The above results are in close agreement with those recorded by Hungerford and further serve to show that it is extremely unlikely that rust mycelium in wheat grains brings about infection of the plant. The mycelium of other fungi is however often present in the developing plant and it might be mistaken for that of a rust.

(*Received March 4th*, 1921.)

SOME PROBLEMS OF ECONOMIC BIOLOGY IN EAST AFRICA (KENYA COLONY)

By W. J. DOWSON,

Royal Horticultural Society's Gardens, Wisley.

(With 1 Text-figure.)

I. Introduction.

The effect of meteorological conditions on the relations between the host and its parasites has received little attention at the hands of the pathologist, but in a country like East Africa it supplies the key to the solution of the problem concerning the severity of attack.

For an understanding of the problems which confront the economic botanist and the agricultural entomologist it is essential to gain some knowledge of the physiographical and climatic conditions of this part of Africa, conditions so varied in extremes as to affect both host and parasite to a marked degree. The Kenya Colony is situated between the 4th parallel N. latitude and the 4th parallel S. latitude, has an area nearly twice that of the United Kingdom, but a population which cannot amount to more than 5 millions. The equator passes through the northern slopes of Mount Kenya, the glacier-covered peak of which rises a few miles south to a height of 17,000 odd feet. 120 miles due south of Kenya and just within Tanganyika Territory is Mount Kilimanjaro, whose summit rises 19,000 odd feet above sea level; and 120 miles W.N.W. from Kenya, and on the boundary line separating Uganda from East Africa lies Mount Elgon, 14,000 feet in height. The snow line is situated at an altitude of 15,000 feet above sea level.

These numbers are apt to give an exaggerated impression of altitude, for the height of all the Central African mountains from the general level of the surrounding country is considerably less because they rise from plateaux and uplands of 5000 to 7000 feet. Thus the highest point of Kenya from the general level of the surrounding country is, roughly, 11,000 feet.

Running north and south right through the country is the Great Rift Valley, one branch of which stretches from Lake Rudolph in the north

to Kilimanjaro in the south. This is the shorter arm of the Great Rift; the other, and longer, passes to the westward and embraces lakes Tanganyika and Nyassa.

The Uganda Railway crosses the Rift Valley at an angle and at the point where it crosses the two escarpments, the western or Mau escarpment is slightly higher (8320 feet) than the eastern or Kikuyu escarpment which is just under 8000 feet (see vertical diagram). Along the western rim of the escarpment between latitude 0 and 1 S. lies the Aberdare range which runs for a distance of 30 miles due north and south, and attains a height of 13,000 feet. The average height of the two escarpments is between 2000 and 3000 feet above the floor of the valley, itself some 6000 to 7000 feet above sea level, where it is crossed by the railway. The drop is always steep and in some places almost precipitous. It will be seen from the diagram that the land rises rapidly from the coast and that

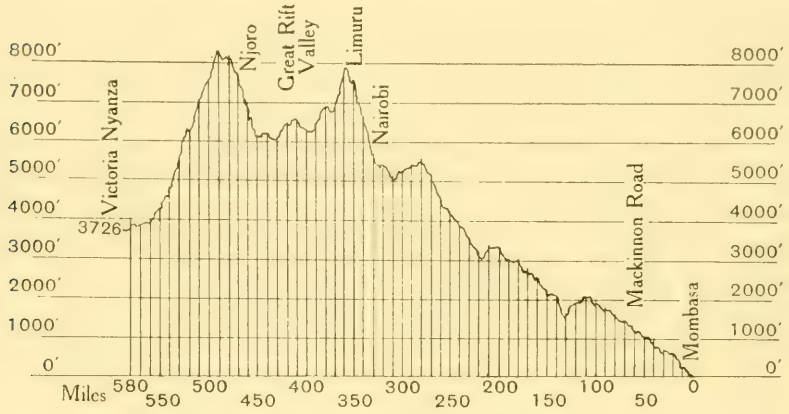

Fig. 1. Vertical diagram of Uganda Railway.

a comparatively short distance along the railway in the direction of Victoria Nyanza the altitude increases very considerably. The meteorological conditions along the course of the railway are very varied. Thus at Mombasa the annual rainfall is between 60 and 70 inches, but 60 miles inland, at Mackinnon Road Station (1300 feet) the average fall is not much more than 10 inches. Again Nairobi (5500 feet) has an annual rainfall of 30 inches, but 25 miles farther along the line Limuru (7500 feet) records an average rainfall of 70 inches. The different climatic conditions at these two places as a result of altitude are remarkable and exert a marked influence upon the spread of fungous diseases.

It is proposed to discuss shortly the most important problems of the

economic biologist, and to consider each plant or crop in the order which one naturally comes across it when travelling on the Uganda Railway from Mombasa to the Victoria Nyanza.

The writer wishes to acknowledge his indebtedness to the Entomological Division of the Agricultural Department at Nairobi for the knowledge acquired concerning insect pests.

II. The Cocoanut Palm.

The rainfall along the coastal belt is between 60 and 70 inches, but towards the S.E. corner in the neighbourhood of Shimoni the rainfall is greater, and besides such indigenous palms as *Hyphaene thebaica*, *Borassus flabellifer* and *Cocos nucifer*, a small patch of the oil palm *Elaeis guineensis* occurs. The rains are distributed in two seasons, a short one in November and a longer period from April to June. Along this coastal strip and for about 20 miles inland cocoanut palms are grown in plantations owned by Swahilis, Arabs, Indians and Europeans. The palms are subject to numerous diseases and pests, the most important of which are undoubtedly the bud-rot or heart-rot, and the cocoanut beetle (*Oryctes monoceros*). In the first, the infection can be traced from the upper external portion of the youngest folded leaf, but whether primarily due to *Phytophthora palmivora* Butl. or to bacteria was not ascertained. The dried and wilted upper portion of the spear contained much mycelium, but towards the actual rotting margin no hyphae were discovered.

Unlike most countries in which bud-rot has been recorded, the palm in East Africa is attacked at the bearing age, seven years, and very considerable loss is thereby caused to owners of large plantations in which several hundreds of acres have been planted out at one time. To the north of Mombasa, on an extensive plantation in which African and Ceylon nuts had been planted, 60 per cent. of the young Ceylon palms were attacked and killed in three years, and so susceptible did these imported palms prove that the rest were taken out and replaced with sisal hemp. The African palms appeared much more resistant, thus indicating that the disease, as it exists in East Africa, is probably native to that country, and has not been imported. Besides the high susceptibility of imported palms and the comparative resistance of native palms, three other points are worthy of notice. One is the striking fact that palms are only found in certain places and have never been known to thrive in others. Thus along the coast south of Mombasa on the Gazi Road, palms grow in patches which alternate with strips of country apparently similar in all other respects but upon which there are no palms.

The palm patches are mostly cultivated, are owned by natives, and alternate with areas of bush, forest or grass. The oldest natives in the district state that their ancestors tried to grow cocoanuts on these places but invariably failed. It is usually only these palmless areas which can be acquired, and in a number of instances it has been found that the cocoanut will not thrive in such.

On the other hand it has frequently happened with plantations of a permanent crop such as cocoanuts, coffee and citrus, that the first European holder of the land, in order to save time and expense, and to ensure a "quick return," has hastily ploughed and planted with immature seed or weak plants. These grow for a time according to circumstances, and to the new-comer, who is looking for a plantation already laid out, they appear in good condition. The original holder sells his plantation at a handsome profit on his outlay and is heard of no more. It is usually later when the crop should be bearing that serious trouble commences, and such cases are some of the most difficult with which the economic biologist is confronted.

The third point which is important in any consideration of the bud-rot disease is the state of cultivation in which the plantation is kept. On a certain plantation in which bud-rot had been recorded since 1912, those palms in the camp for native labour were not only of greater size and of more healthy appearance than those in the plantation, but in addition bore fruit earlier (5–6 years) and never showed a sign of the disease. It was only among the palms outside of the encampment that the disease appeared. These had been planted at the same season, and the only obvious difference between them was in their subsequent treatment. The camp had been kept scrupulously clean, no growth between the huts, and therefore between the palms, was allowed, and rubbish which might collect from food was never left lying about. On the other hand, but a few yards away from the camp, the ground was only weeded occasionally, as the supply of labour allowed, and then only between the palms and not underneath them. The idea seemed to prevail that hoeing up grass and weeds from the spaces between the palms and putting this to rot on top of the weeds growing at the foot of each tree, would produce excellent tilth. In reality, this procedure gave rise to the formation of the most favourable breeding-ground for all manner of fungi and insects —not to mention the non-aeration of the root system.

Among insect enemies, mention must be made of the rhinoceros beetle (*Oryctes monoceros* Oliv.). This large beetle, nearly 2 inches long, flies mostly at night and feeds on the tender tissues of the youngest

unfolded leaf. The edge of the leaf is not eaten away but a cylindrical hole is eaten out, the diameter of the beetle's body, from one side to the innermost soft tissues, passing through each leaflet in turn. Consequently, when the leaf unfolds a circular rent appears in each leaflet, the line of holes being nearly straight. Very often the beetle manages to bore through the midrib, in which case the end of the leaf falls over and rots away. The pest can be so bad that nearly all the leaves present these lines of holes with a number of the tips fallen over. Occasionally the beetle eats the hole near enough to the growing point of the palm as to be the means of starting a heart-rot which in its effects upon the palm is similar to the bud-rot.

Effective measures of controlling this pest were only devised after the life-history of the beetle had been discovered. The female beetle lays her eggs in old rotting stumps of the cocoanut palm or other trees in the vicinity. Hence the idea arose of making beetle egg traps and, as the results have shown, these have proved fairly successful if regularly inspected. The traps are made by collecting all the old stumps and decaying vegetation and packing them in pits in the neighbourhood of the palms. The pits are roughly 2 feet deep and 10 feet across. It is important to destroy all the rest of the rotting stumps and vegetation not used in the construction of the traps, which would be used by the beetles to lay their eggs. At regular intervals the traps are inspected, and when found to contain a goodly number of larvae, a covering of sand is placed over them and carbon bisulphide is injected into each, thus killing the larvae.

III. Sisal Hemp.

Besides the cocoanut palm, sisal hemp, *Agave rigida*, var. *sisalana*, is also grown in extensive plantations along the coastal belt. This plant has borne in the past the reputation of being the only crop in East Africa which possessed no enemies. Sisal was first introduced in 1895 in the form of bulbils from Yucatan by the Germans into German East Africa (now Tanganyika Territory).

In the extremely dry conditions of Yucatan the plant grows for twenty or more years before producing its single inflorescence, after which it dies down. Owing to this slow growth but few leaves are formed each year, and hence only a few can be cut annually from each plant for the decortication of the fibre. In Yucatan it is possible to make use of the waste which is washed away by a stream of water and which contains a considerable amount of sugar. The liquid is filtered and fermented to produce a strong alcoholic drink.

In East Africa owing to the very much moister conditions the plant reaches maturity far sooner and flowers and dies in its fourth year. The leaves, which are ready for cutting at the end of three years, contain very much less sugar, and it was found on analysis that even in the dry season not sufficient sugar was present to render the fermentation of the waste practicable, without going to the expense of concentrating the liquid first. So far, no sisal planter has seen his way to do this, and the subject has received no further attention, though without doubt both sisal waste and coffee berry pulp could be utilised for the production of alcohol.

In a very wet rainy season at the coast the ring-spot disease, due to *Colletotrichum agaves* Cav., has been recorded, and nearly always under such conditions a sun-scorch also takes place in which large irregular patches, red in colour, are produced, rendering decortication difficult or impossible. The spores of *Colletotrichum agaves* can be disseminated by air currents. During the rainy season when this disease appeared at the coastal plantations, a yellow bacterial blotch occurred in a plantation near Nairobi and by the amount of gum produced in the tissues decortication was rendered impossible. The sunken yellow areas were produced on the upper half of the leaves and varied greatly in size, from a small speck to a patch several inches in length. The bacteria entered through the stomata and the organism, a bacillus, was isolated and produced the disease by inoculation, but was not studied in detail. The blotch ceased to spread on the cessation of the rains and is not likely to cause much damage except in a prolonged and abnormally heavy rainy season.

No damage as yet has been caused by insects, but larger beasts such as the porcupine do, in certain parts, cause considerable loss by eating off the entire tops of young plants, thus reducing the length of the leaves and consequently the fibre.

IV. Coffee.

Coffee growing is one of the staple industries of the highlands of East Africa, and has steadily increased since *Coffea arabica* L. was first planted by missionaries a quarter of a century ago. Of native coffee, only one occurs in the country, namely, *C. nandiensis*, which is found on the steep banks of rivers at an altitude of over 7000 feet and is a shade-loving plant. *C. robusta* is the native coffee of Uganda. It is to be noted that there is no resting period such as occurs in Rhodesia, where once every year all the leaves fall from the tree. In East Africa coffee is not deciduous, but is continually producing more leaves, rapidly during the rains,

much more slowly in the dry season. Both *C. arabica* and *C. nandiensis* are subject to a number of fungous and insect enemies. Meteorological conditions play a most important part in the severity of attack of both fungi and insects, particularly so in the coffee leaf disease due to the rust *Hemileia vastatrix* B. & Br.

So far as the writer is aware, coffee cultivation in the eastern hemisphere, including Africa, has always been intimately connected with that of *Hemileia*, and has usually resulted in the ascendancy of the parasite sooner or later. This does not necessarily mean that the coffee trees are threatened with destruction as was the case in Ceylon, but it does mean that *Hemileia* is slowly spreading in all those countries in which cŏffee is grown, with the present exception of the highlands of East Africa.

It is hoped to deal with the subject of coffee leaf disease in Central and Eastern Africa in more detail in a further paper. For the present purpose, some observations of the disease as it is now found in the highlands of Kenya Colony will suffice.

The first observation of importance is that only once in Africa and in Ceylon have the teleutospores of the parasite been found. It is a curious and as yet an unexplained fact that since Marshall Ward worked out the life-history of the fungus in Ceylon, and a German observer reported the presence of teleutospores on some African specimens of the disease not long afterwards, these spores have never been observed since. The likelihood, therefore, of the existence of an aecidial stage on some other plant is not very great. Coffee leaf disease, like many other rusts, is propagated in the countries in which it is found by the uredospores only.

The second observation to be recorded is, that the first attack of *Hemileia* is undoubtedly the most severe; subsequent attacks, other things being equal, are less marked in intensity. Nearly all the trees are badly infected, but in well-kept plantations only a small percentage of the leaves actually fall, although the lives of the others are considerably curtailed. Entire defoliation never takes place, and subsequent attacks are less severe, that is to say, not so many pustules of uredo-sori are formed and not so many leaves are infected. That the general health of the trees has much to do with the severity of the attack is obvious when an ill-kept plantation is compared with others better cared for in the vicinity at any season of the year. The initial preparation of the ground, the proper planting of the seedlings, pruning and the amount of berries the trees are allowed to carry, are all factors which influence the resistance of the host.

The third and perhaps most important observation is the effect of altitude and, therefore, of temperature both on the tree and on *Hemileia*. At the Mission Station of Bura near the coast, at an altitude of nearly 2000 feet, the annual rainfall is about 50 inches and the temperature about 75–80° F. both day and night. The atmosphere is therefore warm and moist, conditions favourable to the luxuriant growth of coffee, but much more so to *Hemileia vastatrix* which has destroyed the coffee plantation attached to the Mission. In the neighbourhood of Nairobi the general altitude of the plantations lies between 5000 feet and 6000 feet, and the rainfall of the district averages 30 inches. The atmosphere is therefore dry, although it is to be noted that very heavy dews are precipitated at night, and it is in this dew that the uredospores usually germinate. The temperature is never very high, and rarely exceeds 75° F., dropping again at night to the region of 50–40° F. and sometimes lower. The atmospheric conditions are warm but not moist and the general balance of conditions is less favourable to the spread of *Hemileia* than to the growth of coffee. Ten miles to the north-west of Nairobi in the Limuru district very different conditions prevail. The altitude is greater, between 6000 and 7000 feet, and hence the climate is colder on the whole. On the other hand, the rainfall is much greater, averaging between 60 and 70 inches. The atmosphere is saturated in the mornings and a "Scotch mist" is the normal experience. The climatic conditions at Limuru, therefore, are the reverse of those 10 miles away, and a moist but comparatively colder atmosphere prevails. Coffee under these conditions is not so luxuriant in growth, is slower but more hardy. *Hemileia* is prevalent throughout the district but is scarce; the first attack is the worst as is the case at lower altitudes, but it is nothing like so severe, and in well-kept plantations in a normal season the rust has to be searched for.

The conditions then which prevail at altitudes of 6000 to 7000 feet are still favourable to coffee but very much less so to *Hemileia* and the limiting factor to the rapid spread of the disease is temperature. At such altitudes the temperature is too low for the parasite to flourish.

It has been pointed out that subsequent attacks of the rust are less severe than the first, which means that the coffee trees acquire a certain power of resistance, or become less susceptible after the initial attack. That this partial immunity is not due to a lessening of the virulence of the parasite is demonstrated by the fact that a hitherto unattacked plantation in the vicinity of others which have been already visited is much more severely infected when *Hemileia* is present on all at the same

time. The virulence of the fungus remains the same, the resistance of the host increases.

Under such conditions, spraying for leaf disease has proved successful at altitudes of 5000 to 7000 feet. Any dilute fungicide has been found by experiment not only to control the disease but, if applied at the right time, to completely eradicate it from plantations. The usual time for spraying is just before the long rains commence, and again at their termination. Reinfection usually takes place in subsequent seasons by reason of wind-blown uredospores from a plantation which has *not* been sprayed. In the Limuru district the disease does such little damage that spraying, always an expensive business, has not been resorted to. The most popular fungicide, and one easily made up, is known locally as "carbide," and is prepared by adding 12 ozs. of calcium carbide to 40 gallons of a solution containing 2 lbs. of copper sulphate in water. At lower altitudes, *e.g.* between 4000 and 5000 feet, spraying with such dilute fungicides is of no avail; but with a stronger mixture containing 4 lbs. of copper sulphate and 24 ozs. of calcium carbide per 40 gallons, the results are much more encouraging, particularly on well-cultivated plantations. At such altitudes it is essential to spray regularly to keep *Hemileia* in check. Below 4000 feet, if the rainfall is at all suitable for the growing of coffee, the other factor of temperature is so much more favourable to the rapid spread of *Hemileia* that the disease cannot be controlled by any known method, and on account of this, coffee growing is rendered unprofitable at such altitudes.

Of other fungous diseases of coffee, mention may be made of the leaf and berry spot due to the attacks of *Cercospora coffeicola* B. & Cke. Considerable damage has been caused by this fungus on neglected plantations, and cases have occurred in which defoliation has resulted. On the berries it is rather more serious from the planter's point of view, as the affected fruits cannot be pulped clean. This trouble is more prevalent during unusually heavy and prolonged rains but can always be found on ill-cared for plantations either on the leaves at any time, or on the berries as they commence to turn red. The disease is easily controlled by spraying either with the "carbide" mixture mentioned above, or with Bordeaux mixture containing 2 lbs. of copper sulphate per 40 gallons.

Quite recently a berry spot has occurred due to infection by a species of *Septoria*, which in its effects on the fruit is similar to that produced by *Cercospora coffeicola*, but it is not known whether this species is identical with *S. maculosa* (Berk.) Cke. recorded on coffee berries from Venezuela. The disease is more common on low lying heavy soil, and is

quite liable to cause considerable damage unless checked by spraying when the berries are still green.

Rot of the roots is not very common, and wherever it occurs usually indicates that the ground has not been properly prepared at the start, and that stumps and roots of native trees have been left in the soil. These are always sources of infection by root destroying fungi, the mycelium of which spreads from the decaying stumps through the soil on to the roots of the coffee trees.

Dieback of the branches is troublesome in certain parts of the country, particularly where the rainfall is more than 45 inches and the soil is heavy. Up to the present this trouble is not fully understood, but among various contributory causes rendering the trees liable to this disease are unhealthy conditions of cultivation, water-logged soil, attacks of *Hemileia*, overbearing, insufficient pruning, and the presence of *Colletotrichum coffeanum* Noack.

Dieback is far more prevalent in Uganda where the general conditions are not so favourable to coffee as they are in the highlands of East Africa. A very singular dieback of the main stem has occurred more than once in nearly every coffee district and has so far baffled any attempts to elucidate its true cause. Nearly every case of the disease was reported shortly after a heavy thunder-storm had passed over the plantations, and was at first ascribed to lightning. Circular patches of trees from 20 to 50 in number were discovered with shrivelled and blackened foliage; and there was always one tree in the centre which was more affected than the rest. The least affected were on the outside, and intermediate stages occurred between. The shoots bearing the blackened leaves were dead towards the tips and for some distance down each shoot, including the main stem, the cortex was discoloured and the cambium disorganised. Unless the affected parts were cut off well below the discoloration in the cortex the trees invariably died slowly back to the roots.

On old specimens which had thus died, the cambium had been replaced by a brown mycelium and very often the fructifications of a *Diplodia* were found on the bark, and always the pycnidia of a *Phoma* and a *Phomopsis*. At one time it was considered that this particular form of dieback was due in the first place to the *Phoma* or to the *Phomopsis*, but the few inoculation experiments which could be carried out did not lend support to this view.

The problem is an interesting one of some economic importance and calls for a more thorough investigation than has hitherto been possible into the relations existing between the abnormal meteorological con-

ditions, the suddenness of the withering, the rapid disorganising of the cortical tissues and the presence of the three parasitic fungi mentioned above.

The insect pests of coffee are more serious than are the parasitic fungi, and mention may be made of at least two which have been computed to cause more annual loss than perhaps any other disease to which coffee is subject. The variegated bug, *Antestia lineaticollis* Stäl., has been known almost from the commencement of coffee planting throughout Africa. The insect punctures all the young growing parts of the tree, but chiefly the very young flower buds which are formed in whorls in the axils of the leaves. The result is a non-formation of flowers and a proliferation of shoots in their place, thus bringing about an almost total failure to set fruit and causing much additional labour and expense in pruning. *Antestia* also pierces and sucks the green berries producing a stain upon the kernels which considerably lessens their market value. As is usual with such insects, spraying either with a stomach poison or a contact insecticide is of no avail. The bug is active and either hides under leaves and crevices, or flies to the ground where it becomes invisible owing to its colour. In the past the usual method of combating this pest was the collecting by hand of the bugs, but recent knowledge of the life-history has indicated a more effective way of controlling the numbers of the insect. The eggs are laid in clusters of a dozen on the underside of the leaves and are normally pearly white in colour. A large number of eggs are not white but grey, and out of these hatch out, not young *Antestia* bugs but minute chalcids. Two species of these have been discovered which parasitise the eggs of *Antestia*, and it has been found possible on the Government Experimental Farm near Nairobi to breed the parasites in the laboratory in such numbers as to completely check the increase of *Antestia*. It is hoped in time to be able to distribute the parasite early enough to those plantations which show signs of the pest to prevent the insect from doing appreciable harm.

Another serious insect pest is comparatively new, having first made its appearance in 1915, and is a species of *Diathrothrips* (*D. coffeae* Will.). This minute insect appeared in the dry season of 1915 in clouds, and, settling upon the coffee trees of a plantation close to Nairobi, sucked every green part almost dry, producing a conspicuous silvery appearance of the foliage. The trees were entirely defoliated, and in some instances killed. From the Nairobi area the pest gradually spread in a north westerly direction and is approaching Uganda. As in the case of *Antestia*, no known spraying fluid is of the least avail, for at the first contact of an

insecticide the great majority of insects fly into the air and hover over the trees in a cloud. They cannot, however, stand heavy rains, and shortly after these commence the pest disappears. The insect appears in very large numbers only in a prolonged dry season and then reigns supreme until the next rains clear it off again. Where it originally came from, its life-history, and what becomes of it during the rains, are problems still awaiting solution.

A third pest, which is often responsible for a large amount of re-planting, is the cut-worm, a larva of various species of the moths *Agrotis* and *Euxoa*. *E. segetum* is the most common. Newly planted out seedlings are very subject to attack and are girdled just below ground level. Gathering by hand, and the protection of the young stem by a band of some durable substance soaked in grease, are the methods employed in controlling this pest. Baits of chopped grass sprinkled with Paris Green have not proved very effective.

The remarks relating to the sound cultivation of the cocoanut palm are equally applicable to coffee and indeed to any permanent crop.

Finally, as regards coffee, it is a point of considerable interest to compare the yield of the East African crop with that of other countries of the East. In the best days of Ceylon coffee the heaviest yield was not more than $\frac{1}{2}$ ton to the acre, and the average was rather below this. In East Africa the average yield is greater; over a ton has been recorded more than once, and $\frac{3}{4}$ ton to the acre is not considered an excessively heavy crop. Latterly, however, it has been found by experience that the trees do better, are not so exhausted and are therefore more capable of withstanding disease, if the crop is limited to not more than $\frac{1}{2}$ ton to the acre. This can be done by stripping off some of the young fruits soon after they have set.

V. The Forests of the Highlands.

The forest areas of East Africa are not very numerous but are of considerable extent, the most important for valuable timber being those of the Highlands at altitudes ranging from 6000 to 8000 feet. Of the many different trees which yield good timber only a few are used at present to any great extent. The yellow wood, *Podocarpus gracilior*, and the so-called East African Cedar, *Juniperus procera*, are used extensively in building. The m'hugu, *Brachylaena hutchinsi*, a giant member of the Compositae, is largely used for fuel, and for fencing and telegraph poles as white ants do not attack its wood.

Podocarpus, so far, has no fungous enemies; its timber, however, is very much attacked by termites. *Juniperus procera* is subject to the attacks of the bracket fungus *Fomes juniperinus* (Schrenk.) Sacc. & Syd., which causes very great damage by producing a heart-rot. About 70 per cent. of the trees are affected, and it is quite common to see newly felled trunks with a large part of the wood replaced by spongy red masses extending for considerable distances. *Fomes juniperinus* is probably a wound parasite, the hoof-like fructifications of which are usually formed just below a small branch which has been broken off a long time previously and which has not been occluded over. The heart wood of *Juniperus procera* is resistant to the attack of white ants.

Brachylaena hutchinsi, a tall tree with a straight trunk, is often killed by strangulation brought about by the entwining and anastomosing branches of a species of fig. This fig gradually surrounds the trunk of its host and all stages are commonly met with, from the partially grown fig at the base of its victim to the nearly fully matured parasite with its rounded head of foliage, out of which can just be distinguished the topmost branches of the *Brachylaena*, evidently in extremis. Finally a stage is reached in which the m'hugu rots away leaving the fig standing alone, and soon afterwards the fig itself dies.

Recently, an important *Sclerotinia* disease of the young seedlings of *Brachylaena* in the nurseries of the Forestry Department near Nairobi has been recorded and partially investigated. The young trees were found to wither and die when they had reached a height of from 3 to 4 feet. An examination of the roots brought to light numerous small black sclerotia, irregular in shape, clinging to the base of the stem just below ground level. The sclerotia varied in size from a rounded mass 1 mm. in diameter to a flat irregularly shaped mass 1 cm. across. Several specimens of the sclerotia were collected and kept under conditions as natural as possible, and after a few months began to produce apothecia on long ($\frac{1}{2}$ in.) stalks. None, however, succeeded in reaching maturity, and when nearly fully opened, withered and died down. On investigation these nearly ripe apothecia were discovered to be infested by eelworm, and the question arises as to whether or not in nature the spread of this fungus is kept in check by the eelworm. The investigation could not be completed and the few apothecia produced in the places where the infected trees had been removed likewise succumbed to eelworm attack.

VI. Citrus.

The lime is found wild along the coast, probably introduced from the east by the early Portuguese settlers, while the excellent greenish orange of Zanzibar was probably planted by the early Arab traders.

At the present time all varieties of citrus are grown, generally from budded stocks imported mostly from South Africa, and at one time it was thought possible to export the fruit to England. Oranges, lemons, grape-fruit, etc. might be placed on the English market at a time when no citrus fruits from other countries were available. Partly because of heavy sea freights *via* the Cape, and still more heavy Suez Canal dues, and also because the comparatively young plantations of the Highlands are still producing rather thick-skinned and not very juicy fruits, the attempt has not yet been made. Those plantations which have been raised from seed bear very much better fruit but take longer to reach this stage, namely, eight years as against three years from budded stocks. Very much better fruit is produced at lower elevations such as 3000 to 4000 feet. A few factories have been started for the extraction of citric acid.

Perhaps the most serious disease, if not the most common, is the foot-rot or mal-di-goma, generally ascribed to the attack of *Fusarium limonis* Briozi, but which is more likely due to bacteria in the first place. The *Fusarium* is probably secondary and gains entrance through the cracks of the bark brought about by the activity of the bacteria.

Various bacterial leaf spots, but not the Citrus Canker of the Gulf States and South Africa, are common and usually make their appearance in the dry season, causing considerable damage by defoliation. This is particularly the case in a neglected grove or one in an unsuitable situation, such as a stiff soil apt to be water-logged in the rains. The most common form which the spot disease takes is large concentric rings of small blisters, hard in texture and brown in colour.

As regards insect pests, citrus in East Africa is an outstanding example of the introduction of pests into a country on the imported hosts. Both the Australian bug, or fluted scale, *Icerya purchasi* Mask., and the California red scale, *Aspidiotus aurantii* Mask., have been introduced in this manner. Considerable loss has been caused by both, but more particularly by the former, which does not confine itself to citrus and will infest almost all other woody plants, as for instance coffee, roses, black wattle (Acacia). No insecticide has yet been devised which will control this scale effectively. Fortunately, however, there is an African

lady-bird beetle, the larva of which will devour the *Icerya* and helps to check to some extent the damage caused by this insect. A resin soda spray has been employed with good results against the *Aspidiotus*, but it is now the endeavour of the Government to destroy all trees infected by the red scale and to replace them with healthy young stock.

VII. Wheat.

Wheat, like coffee, affords another instance of the variable relations existing between host and parasite under different climatic conditions. For the purpose of the present paper it will prove useful to contrast the relation of the rust fungi to wheat in three widely separated countries such as England, East Africa and Australia. In all three countries the same three rusts attack wheat, namely, the black stem rust (*Puccinia graminis* Pers.), the yellow rust (*Puccinia glumarum* Eriks. & Henn.), and the brown or leaf rust (*Puccinia triticina* Eriks.). In England *Puccinia glumarum* is the commonest and most destructive; in Australia *Puccinia triticina* causes most damage because of its very early appearance in the season, wheat when only a few inches high in New South Wales being attacked. In East Africa the greatest destruction is due to *Puccinia graminis*, while in addition to this, *Puccinia glumarum* is very common on certain wheats of Egyptian origin. These two rusts usually, and *Puccinia triticina* nearly always, appear late in the season, generally after the wheat has come into flower. Hence it will be seen that the problem of controlling rust in wheat in East Africa differs from that in the other two countries mentioned. Climatic conditions also influence very considerably not only the growing of the wheat but also the spread of the rust. Thus at Nairobi there are two rainy seasons in the year, during both of which it is possible to grow early maturing varieties of wheat. The fact that it is necessary to employ varieties which mature early, that is, in four to five months, because of the shortness of the seasons, makes it possible to grow with success such varieties as escape the rust attack on account of this character. As an instance of this, the Australian wheat "Florence" may be cited, which at Nairobi was found to mature in four months after sowing. "Florence" is not a rust resistant variety, but when sown early enough escapes the rust attack. This was demonstrated at the Experimental Farm near Nairobi where, during some of the trials, "Florence" and another Australian wheat, "Bobs," were sown in adjacent plots and at the same time. "Bobs" takes between six and seven months to ripen and always falls a victim to the attacks of *Puccinia triticina* and *Puccinia graminis* which make their

appearance generally in the third month after sowing, so that for the last three months of growth "Bobs" has these two rusts to contend with. On the other hand, "Florence" is nearly ready to be reaped when the rust commences, and has reached a stage when the presence of rust can do but little or no damage. Had "Florence" been sown two months after "Bobs" so as to be ripe at the same time as the latter, it would have been attacked and badly damaged.

Another condition which plays an important part in the severity of the attack is the amount of nitrogen in the soil. In England, wheat, being an exhaustive crop, is usually grown on heavy land. In East Africa the great majority of soils contain a larger proportion of nitrogen than in England, and it has been shown by experiment that an excess of nitrogen renders wheat more susceptible to the attacks of rust. A very striking demonstration of this fact was provided unintentionally in a certain large field of wheat grown in the Highlands (Njoro) of East Africa. At a distance of a few hundred yards the wheat appeared brown in colour with the exception of a small green triangular patch in one corner. The brown colour was due to the presence of *Puccinia graminis* and *Puccinia triticina*, while the green patch was nearly free from rust. The wheat was the variety Rieti and had been sown all at the same time, but on the triangular patch flax had been grown the previous season. Following this observation, repeated trials were made at Nairobi with a susceptible wheat such as "Bobs," one block of which was sown on land which had borne a root crop the season before, and another block of "Bobs" on land which had previously carried flax. The wheat on the old flax land was rusted to a far less extent than that on land which had not borne flax. The explanation offered is that flax, itself a very exhaustive crop, removes from the soil that amount of nitrogen which would otherwise render the wheat very susceptible to the attack of rust. That this explanation is probably correct is borne out by the fact that wheat grown upon land which has previously carried beans is so badly affected by rust that in most cases the crop is almost destroyed. In this case the already high nitrogen content of the soil has been augmented by the activity of the nodule forming bacteria of the beans. It has been found that the following rotation of crops is an excellent one and produces satisfactory results: (1) Flax, (2) Wheat, (3) Beans, (4) Flax or Maize.

The attempt to breed rust resisting hybrids of wheat on Mendelian lines has met with a considerable measure of success, some of the selections so produced possessing such desirable characters as early maturity, good milling grain and "strong" flour. Of two of these varieties, Cross

No. 11 is a selection from the hybrid "Early Rieti" and "Red Fife," and has proved highly resistant to *Puccinia graminis* in all parts of the country so far tried. The other resulted from the cross "Egyptian No. 3" and "Nut Cut." "Egyptian No. 3" by itself was found to be very resistant to *Puccinia graminis* but susceptible to *Puccinia glumarum*, while the Australian "Nut Cut" was susceptible to *Puccinia graminis* but resistant to *Puccinia glumarum*. From the resulting hybrid Cross No. 13 was selected and was found highly resistant to both rusts.

VIII. Flax.

Flax, which grows well in the Highlands, is subject to the attacks of numerous enemies, chief of which at the present time is the cut worm, the larva of certain moths. The loss caused by the depredations of this grub are enormous, whole fields being completely destroyed soon after the plants appear above the ground. Any spray which poisons the cut worms also kills the flax, and up to the present no method has been devised of coping with this most destructive pest. As has been noted, newly planted coffee is also very subject to the attentions of these larvae, but in some districts the pest is more prevalent than in others.

The mature flax plant is often attacked by a flax moth which lays its eggs in the ripening capsules so that considerable damage is caused in this way to flax grown for seed.

Of fungous diseases, the wilt caused by *Fusarium lini* Boll. is the most important and is commencing to spread in certain localities. The appearance of this disease is most probably due to imported seed bearing the conidia of the *Fusarium*, and its spread is undoubtedly the result of the present practice of growing two or even three crops on the same land without a break or rotation. Experiments designed to prevent the spread of the wilt by infected seed have been commenced, and although of a preliminary nature, have given unexpected results. As flax seed is extremely difficult to disinfect by the usual method of wetting with formalin solution, or with copper sulphate, by reason of its mucilaginous coat, it was thought possible that the desired result might be obtained by subjecting the seed to the action of formalin vapour. The first experiments were designed to find out what effect the action of the gas would have on the germinating power of the seed. Fresh seed from an uninfected area was treated for 4 hours, and the amount of formalin or the number of tablets was to be increased steadily until a marked effect on the germination of the seed was produced. After each experiment 200 seeds were counted out and placed in petri dishes to germinate. The

first two or three times this was done no effect either on the rate or on the amount of germination was apparent, the results showing 98 per cent. germination in 48 hours. Later, however, with a greater concentration of gas, the rate of germination was accelerated, 98 per cent. germination being obtained in 24 hours. This result was quite unlooked for, and is the reverse of what would be expected. The usual result of soaking seed, for instance coffee, in a solution of formalin (over night in a 0·3 per cent. solution) is to retard its germination from one month to anything up to three months according to the age of the seed. The effect of the gas on the *Fusarium* conidia was not tried owing to the absence of infected material at the time this work was started.

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THE EXPERIMENTAL PRODUCTION OF WINGED FORMS IN AN APHID, *MYZUS RIBIS*, LINN.

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It is well known that most Aphidini are dimorphic in respect of the viviparous parthenogenetic generations, which may be either winged or wingless; but the appearance of either form is determined by factors not yet understood. Failure of the food supply has been suggested by some authorities, but this view is based on general observation rather than on experiment. Börner(1) points out that in autumn *Rhopalosiphum lactucae* produces a host of apterous females whose attack causes the plant to sicken and die, and yet no winged forms appear. He also records some observations on the "hop louse," where a healthy plant produced more alate females than a sickly one.

In 1901 Clarke(2) cultivated the common rose aphis upon cuttings planted in sand saturated with solutions of various salts, and he recorded that a solution of magnesium salts resulted in the development of an abnormally high proportion of winged forms. I have not been able to obtain a copy of Clarke's paper, but his experiment was repeated in 1912 by Neiills(5) with similar results, and has since been amplified and extended by Shinji(6).

Shinji experimented with several species of aphides, and found that by watering the host-plants with solutions of certain substances, the proportion of winged forms produced might be as much as 100 per cent. He also records that other solutions had a contrary effect.

Among the "wing-producing substances" are included salts of magnesium, antimony, nickle, tin, zinc, and also sugar; while the list of "non-wing producing substances" contains tap-water, alcohol, tannin, urea, and salts of strontium, potassium, and calcium, etc. The aphides were susceptible to the treatment for three or more days after birth, according to the species.

In 1920, I had occasion to rear a number of generations of the red currant aphis (*Myzus ribis*) in connection with a statistical inquiry into variation. As the required characters appeared only in the winged female,

it was desirable to obtain a high proportion of these forms in each generation; therefore, I repeated Shinji's experiment. The numbers used were necessarily small, but since my results are not altogether in agreement with his, and as the subject is of some biological and economic interest, they are given here.

The aphid used is a common pest of red currant bushes, and, during the summer, also feeds facultatively on weeds such as *Lamium* and *Galeopsis*. An account of its life-cycle has been published elsewhere (4).

Cuttings of red currant were planted in nurseryman's sand previously washed for 24 hours in tap-water, which, according to Shinji, is one of the "non-wing producing" agents. A single stem mother or fundatrix was placed on each, and the young born were removed within 12 hours to similar cuttings watered either with tap-water, or else with an $m/50$ solution of $MgSO_4$, the latter being about the optimum strength of this salt for wing production, according to Shinji's observations.

Table I.

	First Generation				Second Generation			
	Solution used on host	Number of individuals	Number of winged forms	% of winged forms	Solution used on host	Number of individuals	Number of winged forms	% of winged forms
A	H_2O	24	8	33	H_2O	250	205	82
	$MgSO_4$	31	23	74	$MgSO_4$	60	18	30
B	$MgSO_4$	41	26	63	—	—	—	—
C	$MgSO_4$	14	10	71	$MgSO_4$	32	25	78
D	$MgSO_4$	34	11	32	$MgSO_4$	254	167	66
E	$MgSO_4$	46	2	4	$MgSO_4$	80	37	46
F	$MgSO_4$	21	7	33	—	—	—	—
G	$MgSO_4$	30	12	40	H_2O	40	35	87
H	H_2O	10	0	0	H_2O	80	61	76
I	$MgSO_4$	20	14	70	—	—	—	—
J	H_2O	42	38	90	—	—	—	—

The results of the experiments are given in Table I. They were not carried further than the second generation since the object of the work was not primarily to repeat Shinji's experiments; and it was found that the proportion of winged forms in each strain was high in the first two generations, whether the salt solution was used or not. It will be seen that where $MgSO_4$ was employed for the first generation, the percentage of winged individuals varies from 70 per cent. to 4 per cent. and that the highest proportion of all was obtained in strain J, where nothing but tap-water was used.

A second experiment was made in May. It was necessary, to ensure the continuance of certain strains, to transfer the third or fourth genera-

tion to a second host-plant, and the common Dead-nettle (*Lamium purpureum*) was selected for this purpose. Winged females of four different strains were transferred to potted plants of *Lamium*, watered with a *m*/20 solution of $MgSO_4$. Their young were removed to currant cuttings, which, with the exception of one, accidentally left among a number of other plants watered with H_2O, were irrigated with the same solution. The numbers reared in this second experiment (Table II) were very small, as the mortality after transference from *Lamium* back to currant is high, but the only winged forms produced appeared on the plant watered with H_2O. This was probably due to chance, but it seems

Table II.

		Solution used on host	Number of individuals	Number of winged forms
I.	(*a*)	$MgSO_4$	6	0
	(*b*)	H_2O	7	7
II.		$MgSO_4$	3	0
III.		$MgSO_4$	3	0
IV.		$MgSO_4$	1	0

to show that the presence or absence of magnesium is not the only determining factor in wing production. It is important to have controls in experiments of this kind, for the appearance of a large number of winged forms in the first generation (Table I) might have been attributed to the salt solution used to irrigate the plant, if other strains, reared under identical conditions but without the magnesium sulphate, had not likewise produced a high proportion of these forms. Moreover, the proportion of winged forms among the magnesium treated broods varies considerably. The evidence points to the conclusion that, as regards this species at any rate, the appearance of alate females cannot be attributed wholly to the presence of magnesium salts, although it is quite possible that the aphides may react to metabolic changes in the host plant, induced by an abundance of such salts in the soil.

Gregory (3) found that the proportion of winged to wingless females in the broods of the pea aphid, *Microsiphum destructor*, could be raised by periodically starving the parent during its development. This lends some support to the view that exhaustion of the host-plant leads to the production of winged migrants which will seek fresh food supplies for the maintenance of the race; but it may be pointed out that periods of total abstinence, alternating with periods of normal feeding, would not necessarily produce the same effect as continuous feeding upon an inadequate diet.

Thus the results obtained by Gregory and Shinji are not wholly in agreement, and neither are altogether borne out by observations made in the field. Thus the maximum production of winged forms in many species takes place in the third and fourth generations when food is abundant, and then diminishes in a marked degree, although the apterous forms may continue to feed and reproduce on the same plant for the rest of the summer (4). If some of the winged females are induced to breed on their birth plant, the young, exposed to the same conditions, are as frequently apterous as alate. In 1918, I found that in mixed broods of *M. ribis*, the earlier born individuals were either winged or wingless, while the later born were all winged. This suggested that exhaustion of the parent might influence the form of the young; but this was not confirmed by the experiment of rearing a series of generations, in which the youngest born female of each brood was chosen as the parent of the next (4).

It may also be pointed out that in most species, the last individuals of the whole cycle, the oviparous females, appear just before the leaves fall, and are invariably apterous. It is true that the males in many species are alate, but in the genus *Aphis* they are usually wingless, and in other genera this sex is often dimorphic, winged and wingless forms appearing on the same plant.

Thus the factors controlling the production of winged forms in the Aphidini cannot be considered as determined yet, and may even prove to be in part cyclical.

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PRELIMINARY OBSERVATIONS ON THE HABITS OF *OSCINELLA FRIT*, LINN.[1]

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(With 1 Text-figure and 2 Charts.)

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INTRODUCTORY REMARKS.

In his summary of our knowledge of the frit-fly, Collin (8) indicates that elucidation of the bionomics of the fly in England is very necessary. Most of the data published in recent years are Russian in origin, and probably not applicable to this country.

It was considered advisable to ascertain the relationship between the fly and its environment, therefore the following observations were made in 1919–20 with the view of obtaining evidence as to (*a*) the prevalence of the fly in the field in the different seasons, and (*b*) the host plants additional or alternative to cereals.

Observations on prevalence in the field must be obtained in different localities, over several years and correlated with meteorological conditions, for the periods of maximum prevalence to be fixed within the limits induced by seasonal variations. The fixation of these periods will be of importance if control through partial immunity is sought.

[1] A grant in aid of publication has been received for this communication.

The breeding cages used were all normal with such slight modifications as were required.

I am indebted to the Professor of Forestry for the provision of a laboratory, to Professor Somerville for permission to use apparatus and the part provision of an insectary, and to Mr J. E. Collin for freely placing his knowledge of the frit-fly problem at my disposal.

A. THE PREVALENCE OF THE ADULT FLY IN THE FIELD DURING THE YEAR.

Various authors in England have noted that the flies swarmed in certain months or on certain days—Westwood (28) in July, Ormerod (21) on July 9th, 1888, Theobald (25) in August—while Collin (8) records that "all stages are easily obtained between May and September." Again, MacDougall (18) states that between June 21st and Sept. 29th he collected the fly on many occasions.

A general deduction from the meagre evidence available would be that the fly is nearly always present in the field in the warm season, but that it becomes very much more abundant both when the oat is in ear and when the grain is ripe.

To test the accuracy of this view, a regular series of field observations, to determine the number of flies present on the crop throughout the growing season, was made in 1919 and 1920. In conjunction with these observations, controlled breeding experiments were conducted, to check the field data.

Method of Procedure.

By the courtesy of the Professor of Rural Economy, the observations were made on the University Farm, Sandford-on-Thames. To eliminate, as far as possible, any large influx of flies released by disturbance of other cereal crops, the field selected was one surrounded by meadowland, the nearest cereal crop being a quarter of a mile distant. No information is available as to the normal extent of grass infestation nor as to the migratory powers of the adult fly.

The plan (Fig. 1) indicates the relative areas and positions of the crops grown in this field in 1919–20 and also the position (A) where sweeping operations were conducted[1].

[1] Observations of the Food Production Department in June, 1919, showed that, in this plot, 37·4 per cent. of the plants were badly attacked, 45·6 per cent. were attacked but sending up ears, while only 17 per cent. were not attacked (*in litt.*).

The flies appeared to frequent the ears, in largest numbers, during calm weather, hence the material was collected about 4 p.m., when generally the wind has least velocity.

The sweepings were made with a 12 ins. diameter net, similar to

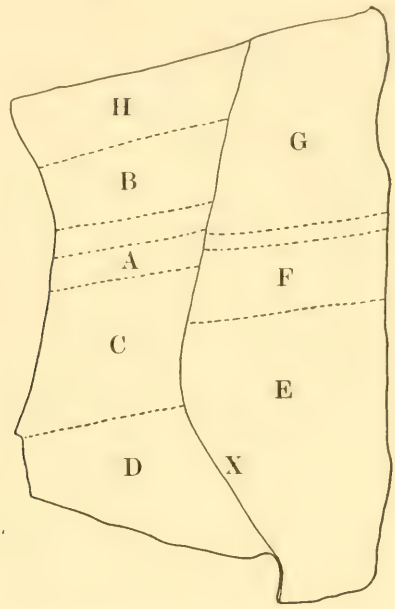

Fig. 1. Field of 41 acres in which sweeping was conducted showing crops carried in 1919–20. Relative areas to scale. Spring oats in 1919 variety "Abundance," in 1920 "Excelsior."

Dates of drilling are prefixed.

A. 2. 5. 19 Spring oats.
10. 3. 20 ,, ,,

B. 28. 3. 19 Spring oats.
10. 3. 20 ,, ,,

C. 31. 3. 19 Spring oats.
15. 5. 19 Rye grass and clover.

D. 30. 10. 18 Winter oats.
11. 10. 19 Winter oats.

E. 1919 Roots.
1920 Winter wheat.
20. 5. 20 Rye grass and clover.

F. 1919 Roots.
4. 3. 20 Spring oats.

G. 15. 11. 18 Winter oats.
1920 Roots.

H. Lucerne and grass.

a marine towing-net, the terminal glass tube greatly facilitating the killing and transference of the material collected. Fifty semi-circular sweeps were made, as far as possible of equal value, over the tops of the oat plants, working up the centre of plot and keeping to the same line on each occasion. When weather conditions permitted, collections were

made on alternate days. To avoid errors in identification[1] the frit material was separated under a binocular microscope.

Data obtained by Sweeping.

The actual figures obtained as a result of sweeping are set out in Tables I and II respectively, as they record the presence of the fly in the field throughout the year except for the winter period October 20th to April 27th, and it is very probable that more diligent search would shorten this period by a week or two.

Table I (1919).

In "Remarks" column, D = sun obscured.
S = sun not obscured.
Number = velocity of wind in miles per hour.

Date	No. of flies collected at A	at B	at C	Remarks
July 5	144	157	112	D 11·0
8	41	50	29	S 3·6
11	308	223	161	S 7·3
13	78	10	34	D 13·2
15	145	105	77	S 10·2
17	1110	364	135	S 4·4
21	168	98	Not taken	D 8·1
23	150	67	,,	D 14·7
25	453	361	,,	D 4·4
27	461	483	155	D 0·7
29	472	468	Not taken	D 2·9
31	127	93	,,	D 10·3
Aug. 2	43	17	,,	D 9·5
5	216	268	114	S 8·1
7	306	Not taken	Not taken	S 5·1
9	161	,,	,,	S 5·9
Aug. 11	345	Not taken	Not taken	S 2·2
13	231	,,	,,	S 7·3
15	685	,,	,,	S 6·6
17	502	,,	,,	D 8·8
20	1437	,,	,,	S 7·3
22	22	,,	,,	D 13·9
24	541	,,	,,	S 5·1
26	56	,,	,,	S 23·5
28	Nil	,,	,,	D 16·9
31	23	,,	,,	S 8·8
Sept. 1	Crop cut			
2	14			From grass headlands, procedure as for oats
9	23			
19	11			
30	26			
Oct. 7	15			
13	4			
19	1			

After Oct. 19th, no captures in field.

Sweepings on B and C were discontinued after August 5th as the figures obtained up to that date were confirmatory to those shown under A.

The abundance of the flies on July 17th and August 20th, after rainy periods, should be noted.

[1] Mr J. E. Collin has informed me (*in litt.*) that he is convinced *O. frit* is the only species attacking oats in England.

Only occasionally was *O. pusilla* Meig. observed in the material collected (which included approximately 12,000 specimens of the frit-fly) and then never in large numbers, nor was this species ever reared from puparia collected from oats in the field. On the other hand *O. pusilla* was bred from barley grains, collected at Garsington, Oxon., the flies emerging on 17. 8. 20. Mr Collin, in confirming the identification, stated that, as far as he was aware, this is the first record of this species attacking cereals in England. Again, this species was bred from field specimens of *Hordeum murinum*, two flies emerging on 13. 10. 20.

Table II (1920).

In "Remarks" column, D = sun obscured.
S = sun not obscured.
Number = velocity of wind in miles per hour.

Date	No. of flies collected at A	Remarks	Date	No. of flies collected at A	Remarks
April 28	1	16·2	June 18	32	D 8·8
30	Nil	11·0	21	168	S 9·5
May 2	11	16·2	23	375	S 8·1
4	5	11·0	26	222	D 7·3
6	1	17·6	28	205	D 11·0
8	19	11·7	30	298	S 11·7
9	48	S 4·4	July 2	76	D 14·7
10	56	S 6·6	3–8	Too wet for sweeping	
11	26	D 8·8	9	38	S 13·9
13	37	S 13·2	12	66	D 14·7
15	12	D 11·0	14	80	D 10·2
19	42	D 11·0	16	293	D 11·0
22	79	S 8·8	19	469	D 12·4
24	18	S 8·8	22	18	D 16·9
27	28	D 8·1	24	38	S 10·2
29	21	D 14·7	26	52	S 9·5
31	7	D 11·0	27	31	S 13·2
			29	102	S 4·4
June 2	75	S 9·5	31	128	S 8·8
4	42	D 11·7	Aug. 3	8	D 13·2
6	50	D 5·9	5	7	D 13·2
8	30	S 8·1	7	31	S 7·2
11	41	S 8·1	9	4	13·9
13	33	D 6·6	12	99	4·4
15	75	S 4·4		Crop cut	

OBSERVATIONS IN THE INSECTARY.

Controlled breeding experiments, with field material, were conducted to ascertain the relationship between the periods of high prevalence in the field and the production of successive broods or generations.

The environmental conditions inside the cages were artificial, excessive moisture and close confinement being unfavourable factors whilst protection from direct sunlight and the presence of a plentiful food supply were favourable factors. Therefore the period between the dates of the infection and the emergence of the flies of the next generation may be only approximate to the natural period.

Method.

Flies were collected in the field on 15. 5. 20 and used to infect various cereals which had been grown under protective covering. In each case

a few plants were potted out, covered with the ordinary chimney cage and kept, for the experimental period, in the insectary. Abundant moisture, and food in the form of cane-sugar, were supplied.

The sexes were easily identified under the binocular microscope, the flies being tubed separately and, when necessary, rendered temporarily immobile by means of a wisp of cotton-wool. The parent flies were undoubtedly of various ages and probably some of the females had completed oviposition.

The data obtained from these breeding experiments are detailed in Synopsis I.

Synopsis I.

Figures in columns 4–8 refer to the number of days after date of infection (15. 5. 20).

Cereal	Height of plant in inches	Parents ♂	Parents ♀	Parents removed	The first sign of attack	Emergence of first fly	Emergence of last fly	Av. emergence No. of flies in brackets
Barley . .	6	10	10	31	14	—	—	—
Spring wheat	3	10	10	31	12	46	53	50·4 (5)
Spring oat .	8	10	10	31	15	45	55	50 (2)
Spring oat .	8	7	8	33	—	46	63	49·2 (4)
Winter wheat	12	7	8		Negative			
Winter oat .	12	7	8	20	3 eggs, larvae failed to emerge			
Maize . .	5	5	5	28	7 eggs on dead sheaths, 4 larvae emerged but failed to mature			
Spring oat[1] .	8	10 (Coll. 24. 5. 20)	10	4	11	41	45	43 (2) Omitted from general average

To avoid confusion between individuals of the consecutive generations, the parent flies were removed from the cages before the emergence

[1] Examination of the cages after emergence had ceased showed that larvae from very young tillers pupated either in the tiller, in the sheath, or in the soil (one case). Larvae in main shoots pupated in the gallery near the top. This is in accordance with field observations, pupation in the soil being rare. In the first cage of spring oats, six puparia were found (flies dead in four) in four main shoots, only one of which showed normal signs of attack. The blades were only partially cut and remained green.

In the case of the barley two puparia (flies dead) were found on examination after 67 days (from date of infection). One larva bored through the stem of an oat plant and pupated on the external surface immediately below the exit hole after 30 days, the fly emerging after 45 days.

A pupal period averaging 16·5 days was noted in the case of larvae in oat stems brought in from the field, potted and caged. In four cases puparia were exposed and the following data obtained:

Larva pupated	8. 6. 20 ♀	emerged	25. 6. 20	*i.e.* after	17 days		
,, ,,	9. 6. 20 ♂	,,	27. 6. 20	,,	18	,,	
,, ,,	13. 6. 20 ♀	,,	28. 6. 20	,,	15	,,	
,, ,,	13. 6. 20 ♂	,,	29. 6. 20	,,	16	,,	

O. frit was bred from Browich wheat grains, flies emerging on 12. 8 20. Samples of eleven other varieties failed to yield the fly.

of their progeny. These flies were caged with new host plants to see whether they were capable of producing another brood. In addition, the first progeny were also caged with separate host plants in order that the period between the emergence of Generations I and II[1] might be determined.

The data obtained from these cages are detailed below.

Thirty of the parents were removed alive 28 to 31 days after the date of the first infection (15. 5. 20) and used to infect oat plants grown under a protective covering. In the three experiments the flies were provided with sugar and water.

(*a*) On the 31st day sixteen flies were placed on the ears of spring oat plants, which flowered on the 32nd day. Copulation between one pair was observed on the 43rd day, eggs being deposited in the glumes on the 45th day. Twelve living parent flies were removed on the 45th day.

Result: no flies emerged and probably the larvae failed to mature owing to lack of nutriment.

(*b*) On the 32nd day, fourteen flies were placed on spring oat plants not in ear. Copulation was observed between one pair on the 38th day and five living parent flies were removed on the 45th day.

Result: the first and last flies emerged on the 69th and 81st days respectively, and the average period was 78·7 days (seven flies).

(*c*) The eleven flies which were bred out about the 50th day were placed on the ears of spring oat, being removed on the 65th day.

Result: the first and last flies emerged on the 82nd and 85th days respectively, the average period being 83·3 days (three males), *i.e.* 33·4 days after the emergence of their parents.

The result of all these experiments is that, from parent flies of Generation III, adult flies of Generation I were obtained after approximately 50 days, and from the same parent flies of Generation III a second series of adult flies of Generation I was obtained in 78·7 days. Also nine of the original parents lived until the 50th day and one until the 59th day, time enough to have produced yet a third brood under favourable circumstances.

[1] In this paper, for convenience of reference only, the generations at present recognised are numbered. I am convinced that further experiment will show that more than three generations may be produced in the field. Generation I refers to the individuals which pass through the stages, egg to adult in spring, Generation II to the individuals passing through the same stages in the summer and Generation III to the individuals which hibernate in the immature stages. In the following spring these individuals produce Generation I.

Again the first brood (Generation I) gave rise to adult flies of Generation II after 33·4 days. *C'—D*, *C'—E* and *D—F* marked on Chart II, p. 115, represent these periods respectively.

Oviposition probably took place soon after 15. 5. 20 and again soon after 17. 6. 20 (the parents being in constant association with plants) as the issue are grouped over two periods, namely, after 50 days (with a maximum of 63 days) and 79 days (with a minimum of 69 days). Therefore the average interval extends to 29 days, which is most probably the interval between the production of separate broods of flies of Generation I. The interval between the emergence of the last fly of the first group and the first fly of the second group is 6 days.

Discussion of Data.

Significance of Prevalence.

The term "prevalence" is used to denote the presence of flies in the field without distinction of brood or generation. Prevalence and emergence are not synonymous terms, as, for a particular brood or generation, the period of prevalence will exceed the period of emergence, by the longevity.

In Charts I and II, pp. 114, 115, will be found smoothed[1] curves representing the prevalence of frit-fly in the latter part of 1919 and in 1920 respectively. Rainfall, maximum shade temperature and minimum grass temperature, also smoothed, are included[2].

The maximum temperature[3] in the sun has a direct influence on the apparent prevalence at different hours of the day, as determined by sweeping, but a much less effect than the more even maximum shade temperature on the prevalence from day to day. Usually frit-flies are

[1] The curves have been smoothed as exemplified here. Taking, for example, the readings for June 2–8, 1920—the adopted means for June 4 and June 6 are the means of readings for the days 2–4–6 and 4–6–8 respectively.

The corresponding means of meteorological data were calculated from the daily readings of the periods 2–6 and 4–8 respectively, because the weather conditions over the whole period would control the total emergence and therefore the prevalence.

[2] I have to thank Dr Rambaut (the Radcliffe Observer) for these figures. This station is separated from the farm by 3½ miles, both having about the same altitude. The error in the farm observations no doubt greatly exceeds the error introduced by utilising the meteorological records of this Station. Self-recording instruments were not available for use on the farm.

[3] Graham-Smith(14) showed that curves for common Muscid flies, caught in traps in the open air, corresponded most clearly to the curve for the maximum temperature in the sun. In this case, however, the number of flies trapped was proportional to the activity of the fly and was not necessarily a measure of the prevalence of the flies at any one time.

more active in warm, sunny and calm weather, but under adverse conditions they seek shelter in the areas protected by the plant foliage, in which areas their activity would presumably be influenced only by the maximum shade temperature. When sweepings are taken on successive days at a definite hour, it is considered that variations in the records, due to fluctuations in the maximum temperatures in the sun, are to a great extent eliminated, especially when the results are meaned as explained above.

Charts I and II show a general prevalence of the fly in the field throughout the warm season together with certain periods of high prevalence[1]. The intervening minima will be real if it can be shown that the absence of flies from the field is not falsely represented owing to the weather conditions being unfavourable for collection.

Considering the minima in succession, first on Chart I and then on Chart II, it will be seen that the weather conditions in the first week of August, 1919, were favourable for collection, the velocity of the wind being about normal (*vide* Table I), rain absent and temperature on the up-grade. One cannot determine the reality of the first two minima in Chart II, as the crops, at this time of the year, show very little growth and collection by sweeping may not be giving very accurate data. About June 8th, however, conditions were good although few flies were caught. On July 7th to 12th conditions were bad, the weather generally being wet, cold, and windy. Yet on July 14th, under conditions much the same as those of July 16th, only one-third of the number of flies was collected. The weather on July 27th is recorded as being sunny and calm, yet the number of flies caught was distinctly low and a similar result was obtained under similar conditions on August 7th. Immediately afterwards, the prevalence curve tends to rise but at this time the crop was cut.

Thus the minima appear to be caused by a real absence of the flies from the field and therefore the frit-fly has certain periods of high prevalence.

Interpretation of the Prevalence Curves.

The tendency in the past has been to conclude that periods of high prevalence coincided with the appearance of successive generations. The abundance of flies has been roughly correlated with the condition of the crop and at the same time associated with the three generations at present recognised.

[1] As the oat crop is nearly always removed before the life-history for the year is completed, one or more maxima will not appear on the Charts.

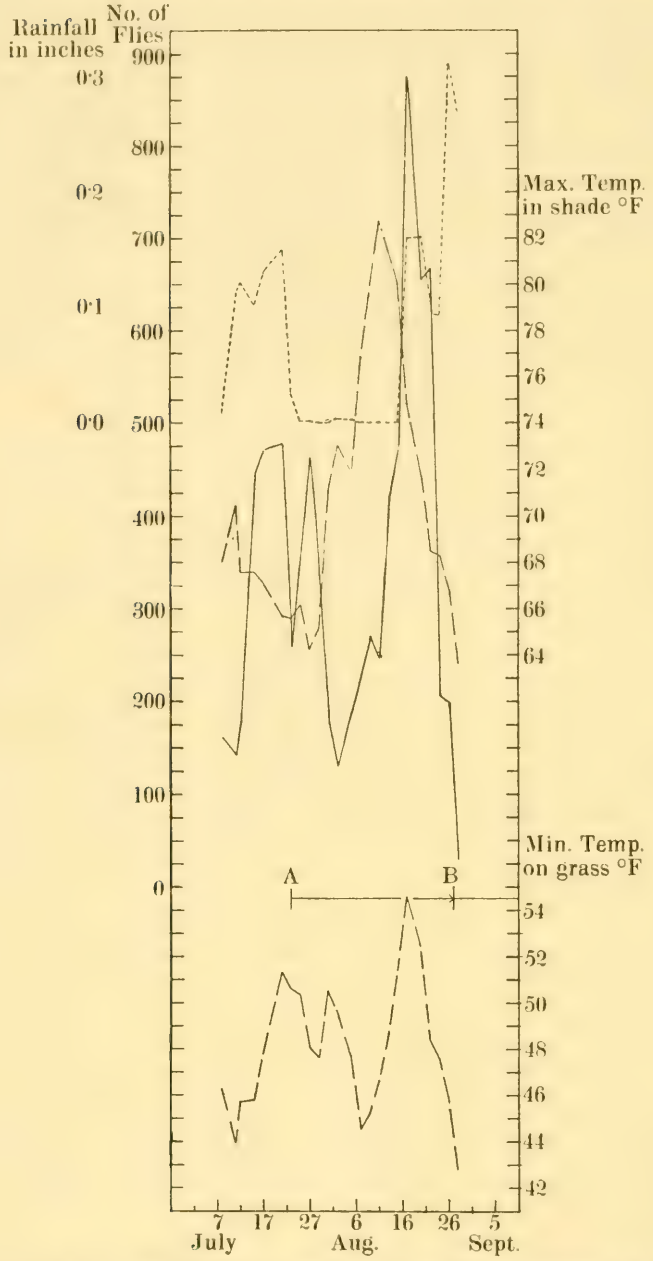

CHART I. Dotted line = rainfall; upper broken line = maximum temperature in the shade; lower broken line = minimum temperature on the grass; unbroken line = prevalence curve. 1919.

A—*B* = average interval in days required for the production of a new generation.

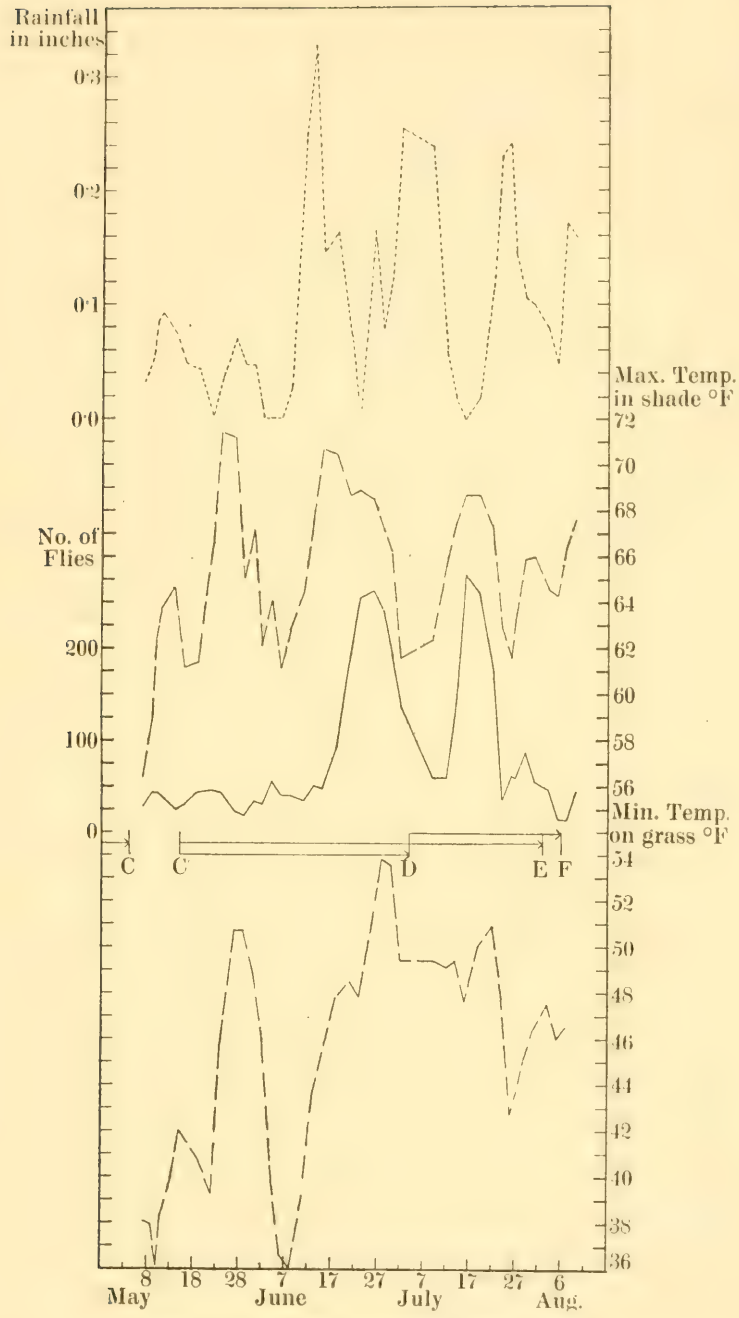

CHART II. See Chart I for key to curves. 1920.

C = time of appearance of flies bred in grasses over the winter.

C'—*D* and *C'*—*E* = average intervals in days required for the production of first and second brood flies from parent flies at *C'*.

D—*F* = average period in days required for the production of flies from the above first brood flies.

The environmental conditions, however, may exercise a very decided influence on the prevalence, most probably masking the effect of the emergence of broods or generations.

For this reason the curves are considered below under two headings, indicating the relationship between (*a*) high prevalence and brood production, and (*b*) high prevalence and environment.

(*a*) *High Prevalence and Brood Production.*

It has been assumed in England that the generation which breeds on grasses over the winter gives rise to another generation which attacks oat stems and tillers, and this, in turn, to a third generation which attacks oat grain.

Quite recently, it has been suggested by Roebuck (1921), that the life-history is more in accordance with continental observations, which, in general, indicate the occurrence of more than three generations. From field evidence, collected over a period of five years, Roebuck concluded that a "brood" is normally interpolated between Generations I and II as generally recognised at present. It is not clear what is meant exactly by the term "brood," in this paper, but there is no doubt that the presence of only two overlapping broods or generations on the oat crop is considered to be very doubtful[1].

When these prevalence curves in Charts I and II are considered in conjunction with the experimental evidence detailed above, it becomes very difficult to believe that the minima of the curves are, in general, closely associated with the imminence of broods or generations. As indicated on p. 112, and marked on Chart II, parents at *C'* gave rise to a first brood at *D* which in turn gave a first brood at *F*; also the parents at *C'* gave rise to a second brood at *E*. Although experimentally the

[1] This conclusion is drawn from the following observations (*vide Ann. App. Biol.* VII, p. 181):

Site of pupa	Earliest gathered pupa	Period of maximum emergence of flies
Grass stems . .	March 25	May, 1st week
Base of oat tillers .	May 21	June, middle
Panicle inside leaf	June 24	July, 3rd week
Oat grains . .	July 31	September, 1st week

Aldrich (1) summarises the life-history in North America as consisting of the winter generation and "following the emergence of this brood as adults in the spring, there are four summer broods." But he does not make it clear whether or no he considers these broods to be equivalent to successive generations. In his tabulated results, he only records that each cage was started "using flies that had emerged a few days before," without stating whether any of the parents were actually bred personally.

It is probable therefore that his figures have reference to broods only and not generations.

broods at *E* and *F* failed to reproduce themselves, in the field they would undoubtedly do so at this time of year. In 1919, when breeding flies on grasses (p. 120), it was found (1) that parent flies at *A* gave rise to flies at *B*, and (2) that either the flies at *A* or *B* gave rise to another series of flies in the middle of September. In the field flies would be constantly emerging.

Under natural conditions moisture is abundant, a sufficiency of food always available and suitable breeding places are provided in succession by the growth of the host plant, namely first the tillers, then the young panicles, and lastly the young grain. In addition wild grasses are always present and a few common species can be utilised for breeding purposes. For example, flies bred from oat stems at *A* would normally have oviposited on the oat panicles, but when compelled, reproduced themselves on the young stems and tillers of grasses[1] and similarly the flies at *D* bred on oat tillers in place of oat grain. All that seems to be required by the larvae is young and rapidly growing tissue and neither the position of the tissue on the plant nor the species of plant (within small limits) is important. Therefore there is no reason to assume that reproduction would be checked by lack of food or host plants.

The irregularity of the emergence obtained experimentally would be highly magnified in natural conditions and the various maxima which appear on the charts will be composed of flies of different broods and generations, the complexity becoming very great towards the end of the season. Although it is fairly clear that the first generation becomes abundant in the first week of June, after this time it has been found impossible to separate the different broods and generations on the charts or to associate them with the high prevalence periods. As the flies are not restricted to particular breeding places, the proportions of the broods and generations at any one period becomes a matter of minimal importance.

It would seem from the experimental data obtained in 1919–20 that three or even four distinct generations may be produced in a favourable season.

(*b*) *High Prevalence and Environment.*

Many factors influence the emergence of an insect breeding under normal conditions in the field. With a phytophagous insect, growth of host plant, rainfall and temperature are probably the more important

[1] *Vide* Section B.

factors. Humidity will have but little influence in the case of the frit-fly as the immature stages are passed in very close proximity to the plant surface, where the humidity is high.

It was expected that the correlation between prevalence and meteorological conditions would be more apparent than is indicated on the charts. There would appear to be some relation between high prevalence and high temperature, and emergence seems to follow periods of rainfall. It is obvious however that insufficient data have been obtained and more are being collected in an endeavour to settle the relationship.

Graham-Smith (13) found that common Muscid flies were very sensitive to meteorological changes, extremes in either direction killing off the flies in large numbers. It has been pointed out above that on certain days, namely July 14th, July 27th and August 7th the flies appeared to be practically absent from the field, although conditions for collection were not markedly unfavourable. It will be shown that, under control, the flies may live for as long a period as 66 days (p. 124), both in spring and summer, an interval much in excess of that between any two maxima shown by the curves. The only conclusion is that longevity in the field must be greatly curtailed by the action of undetermined environmental factors.

The influence of relative preference and parasitism, on the prevalence of the fly on any one crop, has yet to be determined.

Addendum.

The greater abundance of the fly in 1919 is very striking. In the period July 5th to October 19th, 8319 flies were collected as against 3597 flies between April 28th and August 12th, 1920 (3657 flies being collected in July 1919, but only 1391 in July 1920, in an equal number of sweeps).

The results of comparative sweeping on plots A, B and C (*vide* Table I) seem to indicate that the fly normally remains close to the locality of its emergence. This view is supported by the following figures showing the percentages of unattacked plants on the various plots in 1919 and 1920.

1919:	A	17·0	1920:	A and B	30·0
	B	71·3		F	49·2
	C	75·0			

B and C (1919) were drilled on March 28th and A (1919) on May 2nd with the variety "Abundance."

A, B and F (1920) were drilled with the variety "Excelsior" on Mar. 4th to 10th.

In 1919, therefore, crop A presented host plants more suitable for oviposition at the time when the flies were abundant, and this crop was most heavily attacked. In 1920 when the seeds were drilled in at about the same times, the attack was more severe on the land which had been heavily infested the previous year, even though there was no apparent difference in the condition of the crops.

B. HOST PLANTS AMONG WILD AND PASTURE GRASSES.

It has been assumed that grasses are the host plants of the frit-fly during the winter, because frit-like larvae have been discovered in various grasses at this time of year.

Ormerod (21) records, from Continental literature, that "the larvae are found in meadow grasses in summer." The normal utilisation of grasses, as alternative hosts, was evidently not realised. In the previous year it was recommended that oats should be left out of the rotation for a while, in badly infested districts, "as the surest method of all to prevent a continuance of attack." Ritzema Bos (23) records that the larvae live, according to the time of year, in wild or pasture grasses, and Carpenter (6) evidently quotes from similar records.

Edmunds (12) states that he found the larvae in rye grass and also in *Avena flavescens* and *Arrhenatherum avenaceum*, but he does not appear to have bred them through to the adult stage, therefore he may have been dealing with another species of *Oscinella*.

Baranov (3) points out that the larvae hibernate in wild grasses (without mentioning species), and also that the spring generation oviposits on *Phleum pratense*, *Alopecurus pratensis*, *Lolium perenne*, *Triticum cristatum*, *Festuca pratensis*, *Avena flavescens* and *Poa pratensis*.

Criddle (9), referring to *O. coxendix* Fitch, reports, from Manitoba, a severe infestation of cereals on grasslands ploughed in late autumn or spring. But on permanent pastures in England, Cameron (4, 5) and Morris (19) did not find frit-fly. Schøyen (24) noted that the first generation deposited eggs on the leaves of young plants of quick grass and timothy grass. Aldrich (1) in N. America bred flies from larvae which had wintered in *Phleum pratense* and *Festuca elatior*.

Roebuck (1921)[1] records the emergence of flies from *Arrhenatherum* in the first week of May. The possibility of the fly breeding in such common alternative hosts as wild and pasture grasses has therefore

[1] *Ann. App. Biol.* VII, pp. 178–182.

been realised, but very little experimental breeding has been recorded in proof thereof and no attempt has been made to estimate the relative preference for different grasses at different periods of the year.

Method.

Breeding the flies from infected grasses brought in from the field gave uncertain results, since it was almost impossible to obtain pure clumps, except in very few cases, *e.g. Lolium* spp. and *Alopecurus myosuroides*. Sowing seed in small patches in the field was also ineffective. Pot experiments gave positive results (detailed in Synopsis II) with some host plants but without regularity. Negative results, therefore, had very little value.

Samples of common wild and pasture grass seeds were collected and sown separately in 9-inch pots, netted and kept for convenience of observation, in an unheated greenhouse[1], with the lights always fully open.

Flies were reared from field plants, sexed under the binocular microscope, and introduced into the cages. The flies on emergence were easy to observe, as on disturbance they congregated at the tops of the cages.

Two series of plants were infected, one in July and the other in August, 1919[2].

There is no evidence that the flies reproduce themselves without previously securing food supplies, and the longevity experiments (p. 123) indicate that food would probably be sought[3]. If food is essential, the adults in these experiments obtained sufficient from these particular grasses to enable them to reproduce, but probably insufficient to enable them to live a normal period. In 1919, in the cage of *L. italicum*, the last fly died on October 14th and in the cage of *L. perenne*, on October 11th (*vide* Table I for field record).

The parent flies used in these experiments were not more than 5 days old and the majority less.

[1] An insectary was not available at this date.

[2] Negative results were obtained with the following grasses in both series of experiments: *Alopecurus pratensis, Arrhenatherum avenaceum* var. *bulbosum, Avena flavescens, Bromus sterilis, Holcus lanatus, Hordeum murinum, Phleum pratense, Poa pratensis.* Six other grasses also gave negative results, but here growth was not considered normal. Further experiments are being conducted this season with all these host plants in large breeding cages under more natural conditions.

[3] On April 4th, 1920, many flies were observed frequenting apple blossom on trees near the oat crop, and in May and June, on the flowers of *Veronica hederifolia* in the field, apparently feeding. Knuth (15) records *O. frit* as visiting *Potentilla sylvatica, Daucas carota, Matricaria inodora* and *Mentha aquatica.*

SYNOPSIS II.

Series I.

Infection light. Seeds sown 11. 7. 19. Plants infected on 23. 7. 19 when plants 3–5 inches in height. No food supplied. Experiments continued through winter of 1919, no flies being removed from cages. Periods reckoned in days after date of infection.

Host plant	No. of flies used in infection ♂	♀	No. of days between infection and death of last parent	Emergence 1919	Emergence 1920	Average periods
Poa annua	7	9	14	3 flies between 34–47 days	—	39 days
Festuca pratensis	5	7	13	5 flies 31–37 days	—	33 days
Lolium italicum	7	8	18	13 flies 27–40 days;	2 flies on 28. 4. 20	1st emergence, 1919, 32·4 days
				11 flies 61–76 days	—	2nd emergence, 1919, 64 days
Lolium perenne	6	8	17	25 flies 31–47 days;	1 fly on 12. 5. 20	1st emergence, 1919, 41·4 days
				9 flies 62–64 days	—	2nd emergence, 1919, 62·3 days
Arrhenatherum avenaceum	5	5	13	5 flies 29–34 days;	—	1st emergence, 1919, 31·8 days
				1 fly after 63 days	—	2nd emergence, 1919, 63 days

Control experiment for Series I.

30. 7. 19. ♂ and ♀ (emerged from puparia from oat grains) were placed on clean oat panicles and provided with moisture only. Parents dead after 5 days.

Result: The first and last flies emerged after 27 and 35 days respectively, the average period being 31 days (five flies, two ♂ and three ♀). (By an oversight these flies were not supplied with moisture and died without reproducing.)

Series II.

Infection heavy, no food supplied.

Host plant	No. of flies used in infection (bred from infested grain)	No. of days between infection and death of last parent	Emergence reckoned in days after infection 1919	1920	Remarks
Alopecurus agrestis	50	12	—	6 flies between 252–253 days[1]	Sown 15. 7. 19. Infected 21. 8. 19. 1st sign of attack after 22 days
Hordeum pratense	50	15	—	1 fly after 251 days	Sown 28. 7. 19. Infected 23. 8. 19. 1st sign of attack after 27 days
Arrhenatherum avenaceum	50	14	—	1 fly after 263 days	Sown 28. 7. 19. Infected 19. 8. 19

[1] These flies failed to reproduce themselves on the same host plants in the summer of 1920.

The results of the experiments of Series I give a mean of 35·5 days, and this is the average period elapsing between the times of emergence of consecutive generations in the summer.

This period is the same whether the fly breeds on grasses or cereals.

The experiments in Series II indicate an average period of 254 days as the time required for the metamorphosis of the winter generation.

The emergence of a second series of flies, after 63 days, in the last three cages (Series I) is of interest. The average period between the times of emergence of the first and second series is only 27·5 days, too short a period for the time of year (September), to enable one to presume an additional generation. On the other hand, taking the three cases, the greatest length of life of an individual of the first series is 47 days and the shortest interval between the death of the last parent and the earliest emergence in the second series is 43 days; thus it would seem to be a case of either deferred oviposition or the production of a second brood.

It has been proved therefore that frit-fly is able to utilise the following grasses as host plants in the summer: *Arrhenatherum avenaceum*, *Festuca pratensis*, *Lolium italicum*, *L. perenne* and *Poa annua*.

In addition flies were reared in the spring of 1920 from the following grasses which had been infected in 1919: *Alopecurus myosuroides* (= *agrestis*), *Arrhenatherum avenaceum*, *Hordeum pratense*, *Lolium italicum* and *Lolium perenne*.

Addendum.

The following data indicate the extent of the infestation of two common grasses and also that an absence of the typical sign of attack does not necessarily mean the absence of the pest. No decided preference for volunteer oat plants was apparent.

Lolium italicum and volunteer oat plants from plot C (Fig. 1) were collected on 11. 3. 20. Plants were picked at random about every 3 feet, to the number of about 150 for each species, and carefully examined. Control plants produced frit-fly in the first half of May.

	No. of plants	% larvae found	Signs of boring-larvae absent	% of larvae in stems showing no sign of attack	% plants showing typical sign of attack but no trace of frit boring
Lolium italicum	170	8·2	Nil	43	14·8
Volunteer oat	149	10·8	,,	29	9·2

The plants referred to in the last column showed the dead central shoot typical of frit attack, and cursory examination in the field would have led one to suppose that the frit-fly was the cause of the damage.

Actually there was no sign of a central gallery such as is drilled by the frit larva, but only a small lateral cavity, low down on the stem, probably due to the attack of the larva of a *Tipula* sp.

Later in the season (8. 6. 20) *Alopecurus myosuroides* (= *agrestis*) on plots A and B (Fig. 1) was found to be infested to the extent of 89 per cent. (137 plants), the infestation of the oat crop at this time being only 70 per cent. The identification of flies bred from this grass was confirmed by Mr J. E. Collin.

Examination of the winter oats on plot C (Fig. 1) on 23. 3. 20 showed that infection was very light. Nine samples, averaging 55 plants each, were collected at random about every 3 feet and examined by dissection. Only 0·7 per cent. of the plants contained frit-fly larvae and 0·6 per cent. showed signs of boring without containing larvae. On 14. 6. 20, however, larvae were very numerous in the young tillers.

These oats followed a crop of winter oats in 1919.

C. APPENDIX.

Whilst making the investigations previously described, various notes[1] of economic interest were compiled and are presented under the following headings:

(1) The longevity of the imago in captivity.
(2) The value of ploughing as a repressive measure.
(3) The effect of manurial treatment.
(4) Parasites.

THE LONGEVITY OF THE IMAGO IN CAPTIVITY.

As there appear to be no records of the longevity of the adult flies in England the following data is of interest. Observations were made on flies kept at the same temperatures, with and without food and moisture, in the spring and summer seasons.

It may be pointed out that Continental records vary very considerably (from 1 day to 5 months), but the environments doubtless varied also.

Method.

The flies were reared separately, in tubes, from puparia collected in the field, either from oat stems or oat grains according to the season,

[1] To comply with the recent decision of the Publications Committee, this paper has been curtailed considerably, notes on the action of the larvae on the host plant, on copulation and oviposition and on the ratio of the sexes in the field having to be omitted.

the dates of emergence being noted. Individuals emerging on the same days were caged[1] together and sexed after death.

Synopsis III. *Observations in* 1920 (*Spring*).

All cages kept in the insectary.

Conditions under which flies were kept	Date caught in field[2]	Sex	Length of life in days: Minimum	Maximum	Average period
Food, nil	12. 5. 20	♂	3	5	3·5 (4 flies)
Moisture, nil ...	5. 5. 20	♀	3	8	4·2 (32 „)
Food, nil	11. 5. 20	♂	4	6	5 (3 „)
Moisture, abundant	22. 5. 20	♂	3	9	7·1 (12 „)
	11. 5. 20	♀	2	7	5·1 (15 „)
	22. 5. 20	♀	4	9	7 (17 „)
Food, sugar ...	18. 5. 20	♂	6	40	28·6 (12 „)
Moisture, abundant		♀	10	62	44 (29 „)
Flies used in experiments (Section A, p. 109) constantly associated with growing plants and fed on sugar	15. 5. 20	Not sexed	Out of 111 flies: 30 lived until after the 31st day 17 „ „ „ 45th „ 9 „ „ „ 52nd „ 1 „ „ „ 59th „		

Synopsis IV. *Observations in* 1919 (*Summer*).

The last three cages were kept in the shade, 3 feet from ground.

Conditions under which flies were kept	Date of emergence	Sex	Length of life in days: Minimum	Maximum	Average period
Shade near ground	20. 8. 19	♂	5	10	7·7 (10 flies)
Food, nil		♀	8	16	11·2 (4 „)
Moisture, condensation					
Food, nil	22. 8. 19	♂	3	5	4·3 (3 „)
Moisture, nil ...		♀	5	11	5·3 (6 „)
Food, nil	22. 8. 19	♂	6	8	6·6 (5 „)
Moisture, abundant		♀	6	14	11·2 (5 „)
Food, sugar ...	22. 8. 19	♂	29	66	50·8 (5 „)
Moisture, abundant		♀	17	66	50·0 (5 „)

[1] The cages were glass cylinders, with muslin covers, standing on several pieces of filter paper, which were placed on a plate. Food was placed in a muslin bag slung inside and the filter paper kept saturated with water. When moisture was withheld, the paper was omitted.

[2] Some evidence as to the probable length of life of the individual early in the year was desired, but winter crops in this district are not heavily infected and time was not available for prolonged searching for puparia among infected grasses. No doubt the average period is low and the maximum period nearer the true period.

The length of life, under similar conditions as to food supply, is the same in summer as in spring. The presence of an abundant water supply only doubles the life of the individual, whereas a plentiful food supply in addition, ensures a life ten times as long. Under adverse conditions the female is more resistant than the male. It has been shown in Section A (p. 111) that reproduction can take place at least 30 days after emergence.

The longevity of the fly in the field is probably very variable and entirely dependent on meteorological conditions. For this reason no approximation to the longevity in the field can be deduced from the prevalence curves shown in Section A.

The Value of Ploughing as a Repressive Measure.

Collin (8) remarks that "no definite experiments, beyond those of Krassiltchik and Vitkovsky mentioned under 'Pupae' appear to have been made as to how far the fly can be controlled by being buried under the ground in its earlier stages, especially as to whether the larva can complete its feeding and pupate after the affected plant has been ploughed in."

Among the earlier authors there was some confusion of species and control by ploughing was not advocated, but latterly this measure has been recommended by many, *e.g.* Ormerod (21) suggested ploughing "with a skim-coulter attached so as to bury infested plants well down," and Carpenter (6) without any apparent justification goes one step further and says "it is advisable to plough a hopelessly lost crop deeply into the soil, as the maggots and pupae will be killed if buried far down."

Even recently, Schøyen (24) recommends the sowing and ploughing-in of trap crops "as the larvae are thus buried in the ground," although Dobrovliansky (10) had pointed out that it was necessary to roll ploughed-in crops heavily to prevent emergence, having probably seen an account of the experiment of Krassiltchik and Vitkovsky (16), in which they found that flies escaped freely from a glass vessel containing puparia covered with rammed soil, which was kept wet. Some preliminary experiments on a small scale were made in 1919–20 with the view of ascertaining whether the larvae could, under any conditions, complete their development, if buried with the host plant. The results of these experiments are given in the following synopses.

In all cases the presence of larvae in the plants was ascertained by previous examination of the stems, the control plants being treated in exactly the same way.

Synopsis V. *Observations with Larvae.*

No. of exp.	Date of exp.	Host plant	No. of infected plants used	Apparatus	Type of soil used	Condition of soil	Depth of burial of host plants in inches	Situation of apparatus	Result
1	12. 3. 20	*Lolium italicum*	17	Box, 1×2 ft., covered with muslin cage	Loam	Natural	6	In open air; box sunk in soil	Negative; no sign of puparia
2	,,	*L. italicum*	6	Farm drain pipes, 6 ins. diam. netted above and below	Headington sand	,,	6	In open air; pipes sunk in soil to mouth	,, ,,
		Volunteer oat	6			,,	9		,, ,,
3 *Control*	,,	*L. italicum*	8	Muslin cage and plant pot	Plants potted under cage			In open air	6 flies emerged between 54–61 days
4	16. 7. 19	Oat plant	11	Glass cylinder netted above and below	Sieved loam	Atmospheric moisture	6	In open air; above ground in shade	1 fly emerged after 26 days=9·1 %
5	12. 8. 19	Oat grain	28	,,	,,	Moist, then allowed to dry off	9	Laboratory	Negative
6	9. 6. 20	Oat plants	25	As in Exp. 2	Coral rag	Natural	6	As in Exp. 2	5 flies emerged between 26–41 days=20 %
7	,,	,,	,,	,,	,,	,,	9	,,	11 ,, ,, 20–43 ,, =44 ,,
8	,,	,,	,,	,,	Headington sand	,,	6	,,	12 ,, ,, 23–33 ,, =48 ,,
9	,,	,,	,,	,,	,,	,,	9	,,	1 fly ,, after 25 ,, = 4 ,,
10 *Control*	,,	,,	,,	,,	—	—	—	,,	14 flies ,, between 19–38 ,, =56 ,,

Synopsis VI. *Observations with Puparia.*

No. of exp.	Date of exp.	Source of puparia	No. of puparia used	Apparatus	Type of soil	Condition of soil	Depth of burial in inches	Situation of apparatus	Result
11	12. 8. 19	Oat grains	20	Glass cylinder 5 ins. diam. netted above and below	Coral rag	Moistened and sieved in loosely, pressed on top by hand	9	Cylinder sunk in garden soil	8 flies emerged between 10–23 days = 40%
12	,,	,,	20	Glass jar 6 ins. diam. netted above	,,	Saturated and rammed by hand every 3 ins.	9	,,	Emergence from soil, nil = 0% 14 puparia recovered 26. 9. 19 Emergence taken place from 12 ,, failed from 2
13 *Control*	,,	,,	70	,,	—	—	—	,,	56 flies emerged between 6–27 days = 80%
14	23. 6. 20	Oat plants	25	Farm drain pipe 3 ins. diam. observation chimney above and muslin cover below	Headington sand	Dry	9	Laboratory	1 fly emerged after 17 days = 4% 19 puparia recovered 30. 7. 20 Emergence taken place from 13 ,, failed from 2 Dead chalcids found in 4 One fly found at depth of 5 inches, undeveloped
15	,,	,,	11	,,	,,	,,	7	,,	No emergence = 0% Examined 30. 7. 20 4 empty puparia and 2 flies recovered
16	,,	,,	25	,,	Coral rag	,,	9	,,	1 cynipid emerged 16. 7. 20 = 0% Examined 28. 7. 20 16 empty puparia recovered 4 with dead larva recovered 1 with living Hymenopterous larva
17 *Control*	,,	,,	26	,,	—	—	—	,,	17 flies between 3–9 days = 65·5%

The larvae used in Exps. 1 and 2 were practically fully grown and it is not understood why they failed to pupate[1]. Later in the season, under similar conditions, flies emerged freely above ground. In Exps. 4

[1] Saturation of the soil would not kill the larvae for some time although it would probably hasten the rotting of the host plants and thus deprive the larvae of food. The subjoined notes illustrate the resistant powers of the immature stages to an unfavourable environment.

Larvae. Larvae of various ages (a full grown larva averages 3 mm. in length) were collected in the field and submerged, 1 in. deep, in dishes holding about an ounce of water, the dishes not being covered. The water supply was not renewed. A few larvae after submersion were removed and bred to the adult stage in young oat tillers.

Date of submergence	Average length of larva in mm.	No. of days larvae survived submergence		
		Minimum	Maximum	Average
28. 5. 20	2·3	3	15	9·3 (3 larvae)
31. 5. 20	3·0	—	24	24 (1 larva)
26. 6. 20	2·6	6	27	16·5 (11 larvae)
16. 6. 20	2·4	9	21	14·8 (15 larvae)

On 16. 6. 20 six larvae were submerged and they were removed after 9 days, being then placed on young oat tillers. One larva died on the 10th day and another on the 13th day after submersion, but the other four were successful in completing metamorphosis. Pupation took place in 12, 12, 14 and 17 days and emergence of the adult in 29 (♀), 44 (chalcid), 33 (♂) and 29 (♀) days respectively. In another case a larva underwent ecdysis after 27 days submersion, but then failed to pupate.

The larvae were quiescent when submerged, but became active when removed from the water. Death was assumed when no sign of mandibular movement was visible under the microscope.

Ten larvae deprived of food and moisture lived for the minimum, maximum and average periods of 1, 4 and 2·9 days respectively, at room temperature.

Puparia. Twenty-four puparia were taken from oat stems on 28. 6. 20 and divided into batches A and B.

A. Twelve were placed in a dry chimney cage and gave 100 per cent. emergence by the end of 14 days.

B. Twelve were held submerged by cotton wool under water. No emergence took place by the end of 23 days and the water was then removed. After 32 days one female emerged. Three larvae failed to pupate, therefore emergence equalled 11·1 per cent.

The immature stages therefore exhibit considerable powers of endurance. Even the very young larvae are not readily killed. On 3. 6. 20, fifteen larvae varying in size from 0·15 to 0·8 mm. were placed 2 inches from the base of a clean oat plant, on the surface of the soil. After 2 days three larvae were recovered, two from tillers and one from a hole, gnawed in the stem 1½ inches above the soil. The remainder were caught by the greased rim of the pot. When migrating from one tiller to another or from plant to plant, the time of exposure in the field would be short, as they are capable of moving on damp soil at the rate of 1 inch per minute.

and 5 drying off would occur, with the result comparable with that obtained in Exps. 14–16.

When the soil was saturated with water and thoroughly compressed by hand, the emergence was nil (Exp. 12). That emergence of the flies from the buried puparia had taken place was proved by the recovery of the empty puparia. When the soil was loosely sieved in and pressed on top only (Exp. 11), a 40 per cent. emergence was obtained, which was half that of the controls. In the series of experiments with larvae, however, conducted under very similar conditions emergence practically equalled that of the controls.

It will be noticed that whereas flies emerged freely from a depth of 6–9 inches, under normal moisture conditions (Exps. 6–9 and 11), they were unable to make their way a similar distance through a dry medium (Exps. 14–16), the dust from which probably choked their spiracles.

It is probable that only fully grown larvae succeeded in completing their metamorphosis, the young larvae being deprived of food owing to the rotting of the host plants.

It seems fair to conclude that ploughing-in a badly infested crop would fail to control the pest effectively. Subsequent rolling, on heavy land, would probably form a crust of sufficient density to prevent emergence on a large scale, but not on medium or light land.

Ploughing alone would check the development of larvae in their early stages owing to the destruction of the food supply, but would have to be conducted at a time when the attack would not be readily discernible.

Effect of Manurial Treatment.

Vassiliev (27) conducted some experiments in 1911 to show that the application of mineral manures nullified the damage due to frit and concluded that under optimum conditions, produced by the application of phosphorus and nitrogen, frit-fly had no marked economic influence if the degree of infection was limited to 40 per cent. This experiment was repeated last season in its essential features with duplicate plots of area one-twelfth of an acre each, "Excelsior" oats being drilled in on 4. 3. 20. Strips through the centres of the plots were examined, 955 being the average number of plants handled in each case. As the experiment is not original, for convenience of comparison the results are meaned and presented synoptically on the plan adopted by Collin (8), in whose paper Vassiliev's figures are quoted.

SYNOPSIS VII. *Manurial Treatment.*

Rate of manuring per acre in Vassiliev's experiments:

Soluble phosphorus 16 lbs. in the form of superphosphate. Potassium 55·8 lbs. Nitrogen 21·2 lbs. in the form of saltpetre.

Rate of manuring per acre in present experiment:

Soluble phosphorus 37·6 lbs. in the form of basic slag.
Potassium 27·2 ,, ,, kainite.
Nitrogen 23·0 ,, ,, sulphate of ammonia.

The slag and kainite were worked into the ground on Feb. 25th before sowing and sulphate of ammonia was applied as a top dressing on April 22nd.

	Vassiliev's experiment.			*Present experiment.*				
	% of plants attacked	% of plants killed	Yield per acre grain	% of plants attacked	% of plants killed	% of grains damaged	Yield[1] grain	Straw and chaff
Unmanured	43	21	33·7	48·8	9·2	16	27·1	30·9
Phosphorus / Potassium	46	17	37·5	52·2	2·9	14	30·2	30·7
Phosphorus / Nitrogen	35	5·5	47·1	50·4	1·4	14	29·6	37·7
Nitrogen / Potassium	—	—	—	48·8	0·3	10	32·4	37·7
Phosphorus / Nitrogen / Potassium	41	7	49·5	45·8	1·8	14	32·2	37·2
Increase in yield with all manures			47 %			19 % grain / 21 % straw and chaff		
Percentage killed on nitrogen plots			6·2			1·2		
Percentage killed on unmanured plots			21·0			9·2		

The manurial value will only show in the increased yield and the reduced percentage of plants killed. The results of the two experiments are very similar, even though, except for nitrogen, the rates of manuring are different. The percentage of plants attacked necessarily includes plants with attacked tillers, and as these are liable to be infested throughout the season, it is not surprising that this percentage is practically the same on all the plots.

Phosphorus and potassium alone have very little value. The nitrogen plots confirm the accepted opinion that the stimulus of nitrogen tends

[1] The yield in this experiment was the rate per acre, the grain being reckoned in bushels of 42 lbs. and the straw plus chaff in cwts., the figures being derived from the analysis of the contents of circles 4 feet in diameter. I am indebted to Professor Somerville for the provision of the necessary labour and materials for the conduction of this experiment and also for the yield figures.

to limit the damage by decreasing the percentage of plants killed. The percentage of grains damaged was unaffected (the grain in turn being always subject to attack), but on the nitrogen plots the plants were more forward and therefore produced grain and straw of slightly greater bulk.

Although the general infestation was about equal in both experiments the severity of the attack, in the present experiment, was evidently not so great in the first instance, as the percentage of plants killed on the unmanured plots was only 9 per cent. as against 21 per cent.[1] It was to be expected therefore that the percentage increase in yield would also be somewhat less. The cost of application of these manures was not covered by the increased yield.

Advantage was taken of an experiment conducted by Professor Somerville last season to test the relative advantages of sulphate of ammonia, nitrate of lime and nitrolim as nitrogen conveyers, if used for limiting frit attack. The procedure of sampling (average of plants examined per plot, 393) and estimation of yield was as above, and from the synopsis below it will be seen that although nitrate of lime was slightly better than sulphate of ammonia, while nitrolim was much the reverse, none of the three gave a high yield with a low percentage of plants killed. It should be pointed out that sodium nitrate was not available for either of these experiments.

SYNOPSIS VIII. *Treatment with Nitrogenous Manures.*

"Excelsior" oats drilled in on 10. 3. 20, on plots adjacent to one another.

Plots manured 19–21. 4. 20. Rate of manuring per acre in terms of nitrogen: sulphate of ammonia plot, 17·2 lbs.; nitrate of lime plot, 18·2 lbs.; nitrolim plot, 22·4 lbs.

	Percentage of plants attacked	Percentage of plants killed	Percentage of grain damaged	Yield per acre	
				Grain	Straw chaff
Sulphate of ammonia	73·6	11·4	11	36·3	30·2
Nitrate of lime ...	69·2	9·9	15	42·5	31·9
Nitrolim	72·8	10·6	16	29·4	20·5

It is considered that the nitrogen, from the nitrate of lime, was as readily available as it would have been if sodium nitrate had been applied as a top dressing, in place of nitrate of lime. The manures were applied before the flies emerged in appreciable numbers, yet even this early

[1] The soil of the plots on which Vassiliev worked is recorded as being very deficient in phosphorus and nitrogen, which probably accounts for the high percentage of plants killed on his control plots and the high percentage increase in yield.

application was not effective. Nitrogenous manures are not likely to repay their cost when used on average land in England, while phosphorus and potassium alone have no value in limiting damage due to frit-fly.

While collecting these data the degrees of infection were estimated (1) near the hedge and (2) about the centre of plot B (Fig. 1), an average of 328 plants being examined in each case. The percentages of plants attacked and killed near the hedge were 73·5 and 6·7 and in the centre of the plot 61·8 and 1·3 respectively.

Baranov(3) states that the flies attack chiefly on the boundary strips round fields to a width of 9 feet, the central areas being seldom damaged. From the figures given above, it will be seen that this is probably not usually the case in this country. Although the damage in the central areas is distinctly less than on the boundary strips it is still very considerable.

Parasites.

Certain Hymenoptera, not previously recorded as being parasitic on the frit-fly, have been reared from puparia collected for experimental purposes[1]. There is, of course, a slight element of doubt as to the exact identity of the host in such a genus as *Oscinella*, but from the hundreds of puparia collected from oat plants, no other species than *O. frit* emerged. It is practically certain therefore that *O. frit* was the host of these parasites either directly or indirectly.

In the two seasons 1919 and 1920 parasitism was not at all pronounced and also comparatively few *Ichneumonoidea* were collected while sweeping.

Out of one batch of fifteen puparia collected on 16. 7. 19 at random, from oat stems in the field, eight parasites emerged, but on no other occasion was a similar degree of infection noticed, and this batch was probably abnormal.

The few parasites (or hyperparasites) which have been reared are distributed among the parasitic Hymenopterous families as follows: **Proctotrypidae,** one specimen unidentified to date; **Braconidae,** *Chasmodon apterus*, Nees (emerged Sept. 1919); **Cynipidae,** *Psichaera* (Först.) spp. (emerged Sept. 1919); two different species of this subgenus have been reared, and they are of interest because other members of the genus *Eucoila* are known to be parasitic on Diptera; *Aphidius*

[1] I am indebted to Mr G. C. Lyle and the Rev. J. Waterston for the identification of these insects, as far as is possible at present. The latter gentleman hopes to publish a paper shortly on the more interesting species, which are not yet completely identified.

granarius (emerged 16. 8. 20); **Chalcidae,** *Dicyclus fuscicornis*, Walker (emerged Sept. 1919) and specimens of at least two other genera, at present unidentified.

SUMMARY.

1. The adult frit-fly is prevalent in the field throughout the year except for the period November to April.

2. There are periods of high prevalence, which are probably limited by meteorological conditions.

3. High prevalence seems to be associated with high temperatures and emergence with rainfall, and should not be associated with any particular brood or generation.

4. It is very probable that normally four generations are produced in one year and that the fly is double-brooded.

5. The periods between the emergence of successive generations are about 50 days in spring, 35 days in summer and 230–250 days in winter.

6. *Arrhenatherum avenaceum*, *Festuca pratensis*, *Lolium italicum*, *Lolium perenne* and *Poa annua* can be utilised as host plants in the summer and *Alopecurus myosuroides*, *Arrhenatherum avenaceum*, *Hordeum pratense*, *Lolium italicum* and *perenne* in the winter.

7. In captivity, the longevity of the imago averages 50 days in spring and in summer.

8. Ploughing-in and rolling would only control the pest on heavy land.

9. Nitrogenous manures are not likely to repay the cost of application on average land in England.

10. The following Hymenopterous parasites are recorded, for the first time, as attacking frit-fly: *Chasmodon apterus*, Nees, *Psichaera* (Först.) spp., *Aphidius granarius* and *Dicyclus fuscicornis*, Walker.

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A PRELIMINARY SURVEY OF THE SOIL FAUNA OF AGRICULTURAL LAND

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(I) INTRODUCTION.

IT is widely recognised that cultivation has some detrimental effect upon the soil fauna, and various cultural operations are strongly advocated as preventive and remedial measures against the depredations of soil insects. However, it is not known whether arable land possesses a characteristic fauna apart from species peculiar to certain crops, or whether certain species, taking grassland as being the immediate initial condition, are eliminated by cultivation.

Hence it was considered that a survey of the fauna of agricultural land, both arable and grass, might lead to some conclusions as to the effect of cultivation.

A survey is generally attempted either as a piece of taxonomic work or as an ecological investigation. The taxonomic method has become unpopular since it is an inadequate expression of the work, for all the observation and detail obtained end merely in a classified list of names. At the same time the rise of Ecology has tended to change the viewpoint.

Ecology, treating of the general life processes of the animal as opposed to the physiology of organs, has provided an attractive alternative. Unfortunately, however, the subject of Animal Ecology has received

little attention, apart from the valuable work of a small number of investigators. In America something has been done to formulate the principles and methods of animal ecology by Adams, Forbes, Shelford, Vestal and others. In this country almost the only serious attempt at this problem is due to Cameron. The present paper is an endeavour to extend his investigations on soil-insects from grassland to other types of agricultural land.

It is by no means easy to find the distribution in space of the association if the definition of Forbes is accepted (1907).

Cameron has suggested (1916) that a difference in the fauna of two areas possessing different soil-types and vegetative coverings is due to different environmental conditions.

The fauna in an area may exist, however, without any ecological connection between the different species. They may not form an association. If the fauna in two areas come together without any ecological relationship (*i.e.* by chance) it is not possible to state in what degree environmental conditions operate since this chance occurrence may take place under very diverse environmental conditions. Even if an association occurs it may be largely composed of areas of associated species without any ecological connections. There may be also environmental differences within the association allowing of the presence of a different type of fauna. At the same time the species most frequently found seem to occur irrespective of soil-type and the majority of species, being adapted to general feeding, are not restricted to particular plant species or vegetative covering.

If the definition of the association is followed it seems that it operates over broad tracts of country comparable with climatic regions, thus, to the isolated worker to whom the investigation of more than a strictly limited area is impossible, the association has a theoretical rather than a practical value, and it therefore becomes necessary to find some smaller or more practical basis for his research methods.

An attempt therefore has been made in this investigation to standardise the soil-types as far as possible. If instead of taking an area with a varied number of soil-materials, one soil-type is used, more accurate information can be obtained as to the physical condition of the soil, water-content and aeration. In this way the environmental conditions may be expressed to some extent in terms of the soil-type. It is felt that if the results from a number of individual surveys are linked together to cover large areas the solution to the whole problem may be found.

(II) THE SURVEY.

In the conduct of the survey while too much attention cannot be paid to correct soil-sampling, there is entailed something more than the close examination of soil-samples in the laboratory. It is essential in tracing the influence of cultivation to study the survey areas under every weather condition and especially both during and immediately after cultural operations.

In considering suitable localities for the survey areas regard had to be paid to the desirability of the stations selected being at such a distance from the industrial towns as to lessen, to some extent, the effects due to atmospheric impurities; and at the same time to make sure that the fields chosen should be representative of the soil-type in each district.

The survey was carried out on three types of agricultural land.

(*a*) Land continuously under the plough for a number of years.

(*b*) Pasture land, which had been broken up not less than three years previous to the survey. This interval of time was decided upon in order that the life-histories of most of the species present would have been completed at least once, and any effects upon the fauna, which cultivation might possess, would have come into full play after that space of time.

(*c*) Permanent pasture or meadowland providing a "control" to the conditions on arable land and giving some indication as to seasonal variation in the environment.

No. 1 Station, Haveley Hey, is situated $1\frac{3}{4}$ miles south-west of Northenden, Cheshire. The geological formation is the Lower Keuper Sandstone or Waterstones overspread by Drift, composed of sand, gravel and reddish clay with boulders.

The district has an altitude of about 175 feet, and lies comparatively close to the southern bank of the River Mersey. In fact, the height of the river during floods is indicated by the excessive rising of the water-table in the fields, due to the inability of the water to flow away through the drains. The neighbourhood is comparatively open country, but there are a number of small woods scattered over the area.

The Field A ("Moat Field," Haveley Hey Farm, Northenden), chosen as an example of arable land, has been continuously under the plough for many years and is a typical arable soil of that district. The field itself is fairly level, but on the west side slopes down into a hollow in which runs a small stream.

The rotation of crops is as follows: (1) Clover, 1916; (2) second year

ley, 1917; (3) Oats, 1918; (4) Potatoes, Turnips and Mangels, 1919; (5) Wheat, 1920.

The investigation was commenced in October 1919, when the root crop had just been taken from the ground, and the land was in its cleanest condition.

The field received an autumn ploughing at the end of November and beginning of December. The land is a heavy loam and works rather stiffly under the plough on account of the unusually large proportion of the finer fractions of silt and clay. Thus there was a tendency for the soil particles to run together to form hard clods in wet weather.

No farmyard manure was applied to the land previous to ploughing since the wheat followed a root crop. But in the spring of 1919 a heavy dressing of farmyard manure was given preparatory to the root crop. Artificial manures as a top-dressing for wheat were not used, so that any manurial effect upon the soil fauna is due to the farmyard manure alone.

At the beginning of February the wheat seed was drilled without any preparatory harrowing. The field was harrowed to cover the seed, and during the spring the wheat was rolled and harrowed.

To summarise, it will be understood that the minimum of working was done to the land consistent with the proper cultivation of the crop. Any immediate influence which cultivation has had upon the soil fauna is due to the exposing of the surface layer of soil by the autumn ploughing and the working during the early part of the year. Also any indirect effect will be caused by the residues of former crops and of farmyard manure, the accumulation of weeds and fauna introduced by reason of the growth of previous crops in the rotation.

No. 2 Station, Brandlesholme, lies about $2\frac{1}{4}$ miles north-west of the town of Bury, Lancashire. The district is situated upon the Coalmeasures, overlain by Drift consisting of upper sand and gravel.

Brandlesholme forms an elevated tableland about 420 feet above sea-level. On the east lies the River Irwell, to which the land slopes sharply. On the west it declines again to form a hollow in which runs Kirklees Brook. Surrounding the district on all sides, except the south where the town of Bury is situated, are high hills running up into the Pennine Chain. Thus the situation of No. 2 Station is rather exposed.

Two fields were taken as types of arable land ("broken-up" pasture and permanent pasture). These were respectively "Bung's Field" and "Big Meadow" (Brandlesholme Hall Farm, Bury).

Field B ("Bung's Field") was pasture ploughed up in the autumn of 1916 in accordance with the "Ploughing-up Programme" (War-time

legislation). There is a slight elevation in the middle line and the field slopes very gradually to the eastern and western sides.

No regular rotation has been followed upon this field, but the following crops have occupied the land: (1) Potatoes and Turnips, 1917; (2) Oats and Vetches, 1918; (3) Seeds, 1919; (4) second year ley, 1920.

Previous to the commencement of the investigation in October 1919 the "aftermath" of the "seeds" was mown at the end of August 1919. During the winter and spring the land received no treatment beyond the application of a dressing of farmyard manure (10 tons per acre) at the beginning of December.

Unlike Field A, the land surrounding Field B is mostly under grass and not a mixed tenure of corn and grass land.

The soil is a light to medium loam. There is a great abundance of water in this district, but the water content of the soil does not appear to affect its mechanical condition seriously, probably owing to the large amount of coarse sand present.

The effect of cultivation upon the soil fauna in this case does not seem to be so drastic as with Field A. Any direct influence of cultural operations is absent apart from the application of the manure.

Field C ("Big Meadow") consists of permanent pasture or meadowland. The field falls away gently towards the south. There are a number of large ponds or mill reservoirs lying on its western side between the field and the stream in the hollow. There is much water present. At almost the highest elevation the water-supply to the farm is maintained by a wind-engine pumping from a 15 feet well, the greater amount of water flowing in from the upper layers of soil.

As with Field B the surrounding land is under grass. Strictly speaking this land is a meadow since it is mown annually for hay. Haymaking takes place at the end of June. During the winter a dressing of farmyard manure was applied as in the case of Field B. With the exception of "chain-harrowing" in the spring there was no further cultural operations.

Apart from the change in the botanical composition induced by annually mowing the field, it may be considered that Field C is a typical pasture or meadowland and that the same influence upon the soil fauna, found in general to operate in other pastures, will be present.

The investigation of the different areas was carried out from October to May 1919–20. Although it would be desirable that the survey should cover a complete year, there is a certain advantage in practice in making the investigation at that time of the year. Conditions on the whole are much more stable. There is not that constant migration from subter-

ranean and field strata to aerial stratum which takes place during the summer months. Most species of soil-insects are hibernating in the larval or pupal form and the other fauna experience a condition of diminished activity.

In order that a more exact idea might be obtained with regard to the soil conditions, a mechanical analysis was carried out:

Fractions	Diameter of particles	A Moat Field %	B Bung's Field %	C Big Meadow %
Fine gravel	1–3 mm.	2·77	1·69	3·50
Coarse sand	0·2–1 mm.	20·55	44·30	56·40
Fine sand	0·04–0·2 mm.	28·83	17.55	10·33
Silt	0·01–0·04 mm.	11·78	6·19	3·56
Fine silt	0·002–0·01 mm.	15·64	6·50	3·03
Clay	less than 0·002 mm.	12·29	8·19	8·29
Soluble matter and Hygroscopic moisture	—	6·67	10·11	12·98

Soil-samples of a standard size in lines across the field were taken so that representative samples might be obtained. The 9-inch cube sample was used because 9 inches deep is usually the limit of habitable soil, few species being found below that depth and at 9 inches the soil passes into subsoil on most agricultural land.

After taking the sample, the soil was spread out and allowed to dry in the laboratory for two days. It was treated with three sieves of $\frac{1}{4}$ inch, $\frac{1}{8}$ inch and 1 mm. While sifting, the soil both in the sieve and that which had passed through was constantly examined for any specimens. With practice two siftings are sufficient to detect all species, but some dipterous larvae managed to pass the 1 mm. sieve. Little difficulty was experienced by this method in noticing any specimens which feigning death, remained still and harmonised with the soil, because the action of the sifting seemed to bewilder and prevent them remaining motionless.

The specimens were preserved in 5 per cent. formalin, but Dipterous and Coleopterous larvae and pupae were previously boiled. Larvae and pupae, especially those belonging to the Diptera, which could not be immediately identified, were placed in breeding cages in order that the adult form might be obtained.

Special difficulties were encountered in sampling. It was found desirable to let samples dry for a few days since their moist condition in the fresh state prevented effective sifting. Owing to the clayey nature of the Northenden soil it was impossible to take samples in wet weather because the mechanical condition was such as to form hard clods impossible to examine.

(III) THE EFFECT OF CULTIVATION.

In studying the effect of cultivation upon the soil fauna, a comparison of that of grass and arable land in similar areas will be of value, since it may be taken that grassland is the immediate initial condition and that differences demonstrated on arable land are due to environmental conditions induced by cultural operations. It will be seen from an examination of the lists of species attached to this paper that such a comparison leads to several definite conclusions.

(1) The distribution and numbers of the soil fauna are more stable on grass than on arable land. Grassland bears a vegetative covering through a period when little, if any, vegetation exists on arable land. Consequently food material is always present for the large majority of species, since soil fauna are predominantly phytophagous forms. At the same time the land is not cultivated and the hibernation of species or period of lessened activity proceeds normally. On arable land, the opposite is the case, the winter ploughing and working of the soil brings the fauna to the surface and exposes the animal life not only to the harsh climatic conditions but also to bird attack.

(2) There is a corresponding increase in the fauna on both arable and grassland as vegetative growth increases. This circumstance is to be expected since conditions favourable to plants are those most suited to animal life. That this increase does take place on arable land is shown either by the absence or great scarcity of species from the earlier samples taken in winter. It will be understood that the conditions in winter and early spring are extremely detrimental to soil fauna. At that time all circumstances combine to reduce animal life in a drastic manner on arable soil.

It may be said that the fauna of arable land consists of (*a*) Species which have existed from the previous year by passing the winter in the soil; (*b*) Species which are introduced or migrate there in the growing season.

With regard to the first class the survey has shown that at a time when the wintering species were alone in occupation few are obtained in the samples.

The fact that the increase of numbers on arable land always lags behind the increase on grassland, rather indicates that grassland is the source of the influx of fauna on arable land in the growing season. Of course certain species will be attracted to the particular crop and may form the dominant ones for that year. With regard to those commonly occurring it does not seem that their limited numbers on grassland are

sufficient to cause a strenuous competition for food and consequent migration of members of species to less populated areas, such as arable land. The invading species will have at the same time to face the more unsuitable environmental conditions found on arable soil. Accidental migration will take place but will hardly provide an adequate explanation.

(3) There is no characteristic fauna of arable land. The predominant species on arable are those commonly found on pasture. It is not possible in an investigation where only a limited number of species are obtained, to discover whether any of them are particularly susceptible to cultural operations and are eliminated either directly or by deprivation of food material. Even where the absence of some commonly occurring species is shown on arable land the environmental conditions as a whole rather than cultivation as such may provide the true explanation.

It will be understood that owing to the particular nature and uniformity of the vegetation on arable land different species will predominate from year to year. These, however, cannot be considered to form a special fauna since the effect is due to the overwhelming importance of perhaps one factor in the environment, such as the particular crop grown.

The great disadvantage in taking samples to determine the population of an area is that a large error either way must be allowed for in the computation. The tendency in sampling is that dominant species are stressed, and rarer species, since they may occur only in a certain particular section of the area, may be overlooked. The elimination of species by cultivation can only be determined by the comparison of the results from a large number of separate surveys.

I am indebted to Professor S. J. Hickson for the kind interest he has always taken in the work and to Mr R. A. Wardle, M.Sc., Lecturer in Zoology (Manchester University), at whose suggestion the survey was undertaken.

With regard to the classification of soil fauna I have to acknowledge the valuable assistance of Mr H. Britten (Manchester Museum) and the Rev. G. Brade-Burke, M.Sc. (South-Eastern Agricultural College, Wye, Kent).

(IV) SCHEDULES OF SPECIES.

Animal Species.

October 1919—*May* 1920.

(The separate dates in the case of each field represent different soil-samples taken. Where the dates recorded for different species in the same field are identical, those species have occurred together in the same sample.)

(**L**) = Larva; (**p**) = pupa. Where no abbreviation follows the date, the adult form is indicated.)

Species	"Big Meadow" (pasture)	"Bung's Field" ("broken-up" pasture)	"Moat Field" (arable)
Collembola:			
Onychiurus ambulans, L.	—	Oct. 19th (8) 26th Nov. 2nd	Nov. 5th
Coleoptera:			
Notiophilus biguttatus, F.	April 18th	May 6th 8th (2) 12th 13th (2)	May 6th 12th 14th 16th
Leistus rufescens, F.	May 2nd (**L**) 6th (**L**)	—	—
L. fulvibarbis, Dej.	—	Oct. 19th (**L**)	—
Nebria brevicollis, F.	April 4th	May 2nd	—
Loricera pilicornis, F.	—	—	May 16th 17th 18th 20th
Pterostichus madidus, F.	Mar. 7th (**L**) May 6th (**L**)	Feb. 22nd (**L**) April 30th (**L**) May 2nd	May 12th (**L**)
P. vulgaris, L.	April 18th (**L**) 28th (**L**) 30th	Oct. 12th April 27th (**L**) 29th (**L**) May 2nd	May 10th (**L**)
P. strenuus, Pz.	—	April 30th May 3rd	May 4th
Amara plebeia, Gyll.	—	—	May 2nd 14th 16th 20th
Anchomenus dorsalis, Mull.	—	—	May 16th
A. parum-punctatus, F.	—	May 10th	—
Bembidium littorale, Ol.	Mar. 7th	—	May 10th 17th (2) 18th 19th 20th
B. obtusum, Stm.	—	May 8th	—
B. quadrimaculatum, L.	—	—	May 18th
B. guttula, F.	—	—	May 19th 20th
B. lampros, Hbst.	—	May 8th (3) 10th (2) 12th (3) 13th	—
Tachyporus pusillus, Gr	Jan. 4th	—	—
Ocypus cupreus, Rossi	May 2nd	—	—
Philonthus varius, Gyll.	April 18th	—	—
Xantholinus linearis, Ol.	Feb. 22nd (**L**)	—	Nov. 29th (**L**)
Aphodius fimetarius, L.	Feb. 22nd (2**L**) April 18th (**L**)	—	—

Species	"Big Meadow" (pasture)	"Bung's Field" ("broken-up" pasture)	"Moat Field" (arable)
A. prodromus, Brahm.	—	—	May 12th
Geotropes stercorarius, L.	May 10th	—	—
Cryptohypnus riparius, F.	Jan. 4th	—	May 16th
Agriotes obscurus, L.	Jan. 4th (**L**) Mar. 28th (**L**)	Oct. 12th April 29th (**L**)	May 1st (2**L**) 2nd (**L**) 3rd (**L**) 4th (**L**) 6th (**L**) 8th (**L**) 10th (**L**) 12th (2**L**) 14th (**L**) 16th 18th (**L**)
Athous haemorrhoidalis, F.	Jan. 4th (**L**) Mar. 28th (**L**) May 10th (**L**)	Oct. 12th (**L**) Feb. 22nd (**L**) May 6th (2**L**) 8th (**L**)	May 8th (**L**)
Athous sp.	—	—	May 12th (3**L**)
Corymbites pectinicornis, L.	Feb. 22nd (**L**) Mar. 28th (**L**)	May 2nd (**L**) 3rd (**L**)	—
C. cupreus, F.	May 2nd	—	—
Campylus linearis, L.	—	Oct. 26th (**L**) Jan. 18th (**L**)	—
Phyllobius pyri, L.	April 30th (**p**) May 2nd	—	—
Harpalus aeneus, F.	—	May 8th 11th 13th	—
Phaedon tumidulus, Germ.	—	—	May 12th
Apion violaceum, Kirby	—	—	May 18th
Lathrobium fulvipenne, Gr.	—	—	May 8th
Diptera:			
Empis borealis, L.	April 4th (**L**)	—	—
Empis sp.	April 23rd (**L**)	—	—
Onesia sepulchralis, L.	April 23rd (**L**)	—	—
Leptis scolopacea, L.	April 23rd (**p**)	—	—
Bibio Johannis, L.	May 2nd (3)	—	—
Tipula sp.	Feb. 22nd (2**L**) 29th (5**L**) Mar. 7th (**L**) 21st (**L**) April 4th (3**L**) 15th (**L**) 18th (**L**)	Feb. 29th (**L**) Mar. 7th April 4th (**L**) 23rd 25th (**L**) 27th (**L**) 29th (**L**) 30th (**L**) May 6th (**L**) 12th (**L**)	May 2nd (**L**) 4th (**L**) 8th (**L**) 14th (**L**) 16th (**L**) 17th (**L**) 20th (**L**)
Hymenoptera:			
Dolerus haematodes, Schr.	May 2nd (**L**)	—	—
Myrmica laevinodis, Nylander	—	May 6th (3)	—
Chilopoda and Diplopoda:			
Lithobius forficatus, L.	April 18th	—	—
Brachygeophilus truncorum, Bergsoe et Meinert.	April 25th	Oct. 26th	—
Geophilus electricus, L.	—	—	Feb. 25th
G. insculptus, Attenis	Mar. 28th	—	—
Acarina:			
Pergamasus crassipes	—	Oct. 19th	—

Plant Species.

"*Big Meadow*" (*Pasture*). (*Dominant Species.*)

Anthoxanthum odoratum, L., Alopecurus pratensis, L., Phleum pratense, L., Agrostis alba, L., Agrostis vulgaris, L., Holcus lanatus, L., Cynosurus cristatus, L., Dactylis glomerata, L., Poa annua, L., Poa pratensis, L., Poa trivialis, L., Festuca pratensis, L., Bromus mollis, L., Lolium perenne, L., Ranunculus acris, L., Bellis perennis, L., Taraxacum dens-leonis, Dest., Trifolium pratense, L.

"*Bung's Field*" (*Broken-up Pasture*).

Crop-"Seeds" (second year ley): Lolium italicum, A. Br.

Dominant weeds: Stellaria media, Vill., Bellis perennis, L., Taraxacum dens-leonis, Dest., Ranunculus repens, L., Spergula arvensis, L., Senecio vulgaris, L., Plantago major, L.

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ON FORMS OF THE HOP (*HUMULUS LUPULUS* L.) RESISTANT TO MILDEW (*SPHAEROTHECA HUMULI* (DC.) BURR.); V[1]

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Seedlings raised at Wye from seed of the "wild hop" (H. Lupulus L.) *obtained from Vittorio, Italy.*

In previous articles[2] it has been recorded that certain seedling hop-plants of the origin given above are resistant to the attacks of the mildew *Sphaerotheca Humuli* (DC.) Burr. The further observations made during the season of 1920, and recorded below, bring the investigations, which have been continued through seven seasons (1914–1920) to a close. The facts observed during 1920 will be given first, and then a general summary of the results obtained since the investigations were started in 1914.

Observations made during the season 1920.

Cuttings of certain seedlings of the "wild hop" from Italy were grown in pots in the greenhouse and tested for resistance to mildew under the conditions described in previous experiments.

The following table gives a list of these seedlings, together with the number of cuttings ("clone-plants") used.

Not one of these 200 plants, raised vegetatively from 23 different seedlings, showed a trace of mildew on any part throughout the growing season, although inoculated with *conidia* both artificially on several occasions, and naturally almost continuously by currents of air carrying *conidia* from several hundred mildewed hop-plants closely surrounding them.

Some of the hop-plants surrounding them were cuttings of other seedlings of the same origin as the 23 "immune" seedlings noted above.

[1] A grant in aid of publication has been received for this communication.

[2] See *Annals of Applied Biology*, VI, 293–310 (1920), where a complete Bibliography is given.

Table I.

List of Seedlings showing Immunity to Mildew, in the greenhouse, throughout the season of 1920.

Ref. no. of seedling	No. of clone-plants	Ref. no. of seedling	No. of clone plants
V 91	6	OA 49	7
V 92	6	OB 34	32
V 93	14	OD 19	9
Z 2	10	OR 38	28
Z 14	10	OR 39	27
Z 17	5	OY 18	1
[1]Z 20	2	[1]HH 20	2
Z 22	8	HH 44	5
Z 25	10	[1]II 13	2
Z 31	1	II 24	2
Z 42	9	II 30	2
OA 34	2		
	Total	200	

In both cases the cuttings had been potted up in the same kind of soil and grown under the same conditions. The former plants, however, became naturally infected with mildew by *conidia* disseminated in the air of the greenhouse. In those cases where an asterisk is placed in the table given below, the seedling showed extreme susceptibility; in the other cases the susceptibility shown was normal[2].

Table II.

List of Seedlings (of the same origin as in Table I) showing Susceptibility to Mildew, in the greenhouse, throughout the season of 1920.

Ref. no. of seedling	No. of clone-plants	Ref. no, of seedling	No. of clone-plants
*Z 24	8	OA 35	2
*Z 26	9	OA 36	1
Z 39	8	OA 53	1
*Z 41	9	*OB 48	1
Z 54	2	OD 16	1
OA 25	3	*OD 18	4
*OA 26	3	II 31	1
	Total	53	

[1] These seedlings had not previously been tested; the remaining 20 seedlings had shown the same resistance in previous seasons.

[2] As further evidence of the severity of the inoculation to which the "immune" seedlings were exposed, and of the presence of suitable conditions for the growth of the mildew, it may be mentioned that 63 cuttings of various commercial varieties of hops stood in the same greenhouse and that all these became naturally infected within three weeks.

All the above 53 "clone-plants"—cuttings taken from 14 seedlings—became fully infected, and the mildew persisted on them until the end of the growing season.

The cuttings of both the "immune" and "susceptible" seedlings enumerated above were all taken from the parent-plants, growing in the Experimental Hop-garden at Wye College, in the winters of 1917, 1918 or 1919. In those cases where cuttings were taken in successive years, no change from immunity to susceptibility, or *vice versâ*, was ever found. The 200 immune plants mixed in among the other seedlings (of the same origin) white with mildew afforded the most convincing proof of the different "constitutional" characters possessed by seedlings of a wild plant. In many cases, *e.g.* with the cuttings of Z 24, Z 26, Z 41, OA 35, OD 18, II 31, the "susceptible" parent-plant has been grown in the hop-garden by the side, and within $3\frac{1}{2}$ feet, of the "immune" parents (Z 25, Z 42, OA 34, OD 19, II 30), so that all these parent-plants have thus been subjected to exactly the same soil, cultural, manurial and weather conditions. It is clear that the differences in susceptibility shown are inherent in the particular seedlings and not induced by any special environmental conditions.

The phenomenon of "semi-immunity[1]" was again shown by a few seedlings when exposed to infection in the greenhouse. The details are given below.

Table III.

List of Seedlings (of the same origin as in Tables I and II) showing "semi-immunity," in the greenhouse, throughout the season of 1920.

Ref. no. of seedling	No. of clone-plants
Z 15 (semi-immune)	2
Z 23 „	4
Z 38 „	1
OC 6 „	4
BB 5 „	1
OA 33 (approaching semi-immunity)	4
OD 17 „ „	4
	20

The seedling Z 38 had not before been tested in the greenhouse; the other seedlings had shown "semi-immunity" in previous seasons.

A *résumé* may now be given of the facts observed from 1914 to 1920 as to the susceptibility to mildew of the seedlings of the wild hop. From

[1] Described in *Annals Appl. Biology*, VI, 302 (1920).

1914 onwards 480 seedlings have been kept under observation, first as 1- or 2-year-old seedling plants in the greenhouse, and secondly as older, flowering plants planted out in the Experimental Hop-garden at Wye College, where they have been cultivated (trained, "cut," etc.) and manured according to the usual practice in a commercial hop-garden[1]. In the greenhouse the plants were subjected to the severest test by being inoculated naturally almost continuously with *conidia* from surrounding heavily-infected plants, as well as being occasionally artificially inoculated, with the result that, except for the seedlings noted below, they all became severely infected with mildew. In the Hop-garden natural inoculation was relied upon (except in one experiment) to test the degree of susceptibility of the mature plant. Under the conditions prevailing, this method was found to be satisfactory. Mildew was present generally in the hop-garden (at the time when observations were made on the seedlings) in each season from 1916 to 1920, and particularly severe outbreaks of mildew occurred in 1916, 1919 and 1920. Owing to the late-flowering habit of the wild hop, and consequent prolonged period of growth, it was found that October was the best month for examination as to the incidence of mildew. In the case of the ♀ plant, the production at the end of August and during September of the female inflorescence ("burr") and young developing hops provided the best possible infectible material. With the most susceptible seedlings the attacks were so severe that season after season the greater proportion of the "hops" (cones) were deformed by the mildew, or not infrequently the crop of "hops" was entirely destroyed, the female inflorescences being permanently arrested in development and changed into white, hypertrophied, knob-like growths. With the ♂ plant, the infectible material consisted for the most part of the leaves of the axillary side-shoots which developed during late-summer from the lower portion of the main stem ("bine"); where vigorous side-shoots were produced, these provided excellent infectible material, but in those cases where they were absent, as happened with a few plants in certain seasons when they "ripened off" early, the susceptibility could not be ascertained. With very susceptible seedlings, branches of the male inflorescence sometimes became mildewed.

In view of the impossibility of recording in detail the behaviour of all the 480 seedlings, a selection has been made of 52 seedlings, whose records are given in Table IV. The explanations of the signs used are as

[1] Owing to exigencies of space, the seedlings could not be planted all together, but had to be placed, usually in large blocks, but sometimes singly, throughout the Experimental Hop-garden of 2¾ acres.

Table IV.

Ref. no.	Sex	1916		1917		1918		1919		1920	
		H	G	H	G	H	G	H	G	H	G
V 91	♀♂♂♂♂♂♂♂♂♀♀♀♀♀♂♀♀♀♀♀

follows: H and G indicate respectively that the plant was growing either in the hop-garden or, in a pot, in the greenhouse. O signifies that the plant remained absolutely free from mildew on any part of it throughout the season. — indicates either that the plant was absent, or that no record exists—sometimes because no observations were made or more commonly because no infectible material (with ♂ seedlings in the hop-garden) occurred. It was not found possible, as a rule, to estimate the degree of susceptibility shown in the greenhouse; the letter S stands for "normal" or "full" susceptibility; in a few cases, marked S*, the plant became so virulently infected, that extreme susceptibility appeared to be indicated. In the hop-garden S^1 indicates that only a minute trace of mildew occurred; S^2 that the infection was of medium intensity; S^3 that the infection was very severe,—those cases where the entire crop of hops was destroyed by mildew being indicated by the added sign †.

The 52 seedlings, whose annual records are given in Table IV, give evidence as to certain facts which will now be discussed.

(*a*) *Immunity under greenhouse conditions.*

Twenty-seven seedlings have shown persistent immunity to mildew when grown in the greenhouse. The history of the behaviour of these plants in the open, indicated in Table IV, will now be considered in detail. The examination of the plants was made each year in October (see above, p. 149).

Ref. nos. V 91, V 92, V 93. These three seedlings, as soon as raised from the seed, showed complete immunity in the greenhouse throughout the seasons of 1916 and 1917. They were planted out in the hop-garden in November 1917, and in October 1918 all three plants[1] showed a minute trace of mildew, in the case of V 93 consisting of a tiny patch of mildew on the under-surface of one leaf only. In 1919 and 1920 V 91 and V 92 remained free from mildew; V 93 in 1919 had again a tiny "powdery" patch of mildew, at the back of one leaf only, while in 1920 it was free.

Ref. no. Z 2. This seedling was originally planted out in the hop-garden in 1914. Cuttings taken in 1917–18, 1918–19 and 1919–20 proved persistently immune in the greenhouse, and no mildew was observed on the parent-plant in the hop-garden during the years 1917–20, when infectible material was present each autumn, although its neighbour[2]

[1] All three seedlings were then about 5 ft. high and non-flowering.

[2] In every case where reference is made to a neighbouring plant (*e.g.* Z 16 next to Z 17, Z 24 and Z 26 next to Z 25, and so forth) it is another seedling of the same origin. The seedlings were planted 3 ft. 6 in. apart in the row, and the lateral shoots of neighbouring plants frequently became intertwined.

Z 1 (likewise a ♂ seedling) was infected to the extent of S^2 in 1917, 1918 and 1919, and S^1 in 1920.

Ref. no. Z 14. In the open slight susceptibility was shown in 1918 and 1920; no mildew occurred on the plant in 1917 and 1919, when its neighbour Z 15 became mildewed to the extent of S^2.

Ref. no. Z 17. In 1917 this seedling remained free from mildew, while Z 16 was mildewed to the extent of S^3. In other seasons there has been no opportunity of testing Z 17 in the open.

Ref. no. Z 20. This seedling was planted out in 1914; two cuttings taken in 1919–20 proved persistently immune in the greenhouse. In the open the plant was recorded as S^2 in 1917; in 1918 as having a trace of mould on leaves and hops; in 1919 it remained free from mildew, notwithstanding the fact that lateral shoots of Z 19, bearing very mildewed hops, had twined round its stems. In 1920, Z 20 showed a mere trace of mildew on the leaves, but several hops were infected with mildew at the extreme tip—the susceptibility being estimated as being between S^1 and S^2. There can be no doubt that Z 20 has been severely tested in the open, since its neighbour Z 19 has been smothered in mildew each season and in 1920 had its entire crop destroyed.

Ref. no. Z 22. This seedling remained free from mildew in 1917 (when Z 23 was recorded as S^2); in 1918 and 1919 the amount of mildew on Z 22 was S^2 on the hops and very occasionally a trace of mildew on the leaves; in 1920 there was a full crop of hops, *i.e.* none was deformed by attacks of mildew, but very occasionally the mildew occurred on the peduncles—the susceptibility being estimated as being between S^1 and S^2.

Ref. no. Z 25. The history of this seedling is one of the most interesting. It was planted out in the hop-garden in 1914. In 1916 and 1918 it remained free from mildew, although Z 24 and Z 26 on either side of it were smothered with mildew, Z 24 in 1918 having all its hops destroyed by mildew. In June 1918 young leaves of the parent-plants Z 24, Z 25, Z 26 in the open were artificially inoculated with *conidia*; infection resulted on Z 24 and Z 26, while Z 25 proved immune. In 1917 Z 25 remained entirely free from mildew, notwithstanding the fact that several strong lateral shoots of Z 24 had become intertwined with those of Z 25 and had produced very mildewed hops which became intermingled with the "immune" hops of Z 25. In 1919, at the beginning of August, after a spell of abnormally dull, cold weather, a few small patches of mildew occurred on a few of the young leaves of Z 25. These mildew-patches died away completely as the weather became normal (hot and sunny) and no more mildew occurred on the plant. In October

of the same year when the plant was examined minutely all over, no trace of mildew could be found on leaves or hops, although some of the lateral shoots of Z 26, bearing mildewed hops, had twined round the stems of Z 25. In 1920, on August 28, Z 25 was free from mildew; when examined in October there was no mildew on the leaves, but a trace of mildew occurred on the hops, particularly at the tips. In 1920 both Z 24 and Z 26 were so severely infested with mildew that all their hops were destroyed.

Ref. no. Z 31. So far as this seedling has been able to be tested in the open, it shows susceptibility to the extent of S^1.

Ref. no. Z 42. In the two years, 1917 and 1918, when this seedling could be tested, it remained free from mildew, while Z 41 on one side was S^3, and Z 43 on the other side was S^2.

Ref. no. OA 34. In 1917 and 1918 the amount of mildew present (on the hops) was recorded as S^2; in 1919 and 1920 there was only a mere trace of mildew (also on the hops). The neighbouring plant OA 35 was recorded as S^3 each year, and in 1918, 1919 and 1920 was so severely attacked by mildew that the crop was destroyed.

Ref. no. OA 49. This seedling remained free from mildew in 1917, 1918 and 1919; in 1920 there was a mere trace of mildew on a few hops in an otherwise completely healthy crop. In 1919 a cutting was grown in the greenhouse and proved immune; on July 5 this cutting was taken out of the greenhouse and placed in the open air. The plant produced a fresh shoot, which by September 30 was 1 ft. 8 in. high; round this shoot the stem of another hop-seedling which bore mildewed leaves had twined, but OA 49 remained immune.

Ref. no. OB 26. No opportunity has yet occurred of testing this seedling in the open.

Ref. no. OB 34. The only time that mildew has occurred on this seedling was in October 1918, and then only one "hop" was found infected. This single hop-cone had been attacked when in an early stage of development and had been converted into a knob-like growth (white and powdery with the conidial stage of the mildew). This mildewed "hop" occurred in the midst of a normal crop of healthy hops.

Ref. no. OD 19. This seedling remained free from mildew in 1917 and 1918, notwithstanding the fact that in both seasons OD 18 was mildewed to the extent of S^3, the crop being destroyed by mildew in 1918. In 1918 a strong lateral shoot of OD 19 climbed round the stem of OD 18 and produced there perfectly healthy hops among the excessively mildewed hops of OD 18. In 1919 OD 19 showed a trace of

mildew—S[1]. In 1920 there was no mildew on the plant on August 31; in October a trace of mildew was found on one hop only.

Ref. no. OE 14. A trace of mildew occurred on the hops in 1920.

Ref. no. OR 38. This seedling appears to be the most susceptible in the open of all the seedlings which show immunity in the greenhouse. As a seedling plant in 1914 it showed persistent immunity in the greenhouse; it was planted out in the hop-garden in 1915. In 1916 its main crop of "hops" was free from mildew, but a late, young stem, which had run up the old stems to nearly the top, bore several leaves and one "hop" with patches of mildew on them. In 1917 there was a fair amount of mildew on the hops (both in the conidial and in the perithecial stages), chiefly on the peduncles but also on the bracts and bracteoles. In 1918, on September 7, there was a fair amount of mildew on the young leaves, and in October, when the whole plant was minutely examined, the hops generally were found to be severely attacked by mildew, a large percentage being undeveloped or deformed through its attacks. In 1919 there was only a trace of mildew on the hops. In 1920 there was a minute trace of mildew on the leaves and an attack of medium intensity on the hops. In the greenhouse OR 38 has been tested on a considerable scale by means of "cuttings" taken in the four winters 1916–1919 from the parent-plant in the hop-garden. In all, 42 "clone-plants" have thus been exposed to constant inoculation in the greenhouse, and with the exceptions noted below, all have proved persistently immune. In 1917 a phenomenon occurred which had not been observed before and which has not been observed since. On two cuttings of OR 38 in the greenhouse, a minute patch of mildew appeared, on one leaf only of each plant—in one case following artificial inoculation, in the other case, without; these mildew-patches appeared in May and soon died away without the mildew spreading on the plant. The two plants concerned, together with three other cuttings of OR 38 stood in the greenhouse for the rest of the season of 1917 and in spite of constant inoculation with *conidia*, all the plants remained persistently immune. It was clear that the phenomenon consisted of the sudden appearance of strictly local and temporary susceptibility (induced by, at present, unknown causes) and was not a general breaking down of the immunity of the plant[1].

Three cuttings of OR 38 which had remained persistently immune in the greenhouse in 1917, were planted out in the hop-garden in the winter of 1917–18. These three clone-plants of OR 38 have behaved

[1] No other "immune" seedling has ever shown in the greenhouse any signs of breaking down.

as follows. (In 1918 the plants were from $1\frac{1}{4}$ to 5 ft. high and non-flowering.)

Ref. no. W 56. 1918: A fair amount of mildew (S^2) on the leaves. 1919: A trace only of mildew, one hop being destroyed by mildew, and one hop having mildew on the peduncle—otherwise a crop of healthy hops; no mildew on the leaves. 1920: On August 29 there were numerous patches of mildew on three oldish leaves but not elsewhere; in October there was a fair amount of mildew (S^2) on the hops.

Ref. no. Y 28. 1918: Very mildewed (S^3) on the leaves and stems. 1919: A trace of mildew on two hops only. 1920: At the end of August there were a few patches of mildew on one middle-aged leaf only; in October there was a fair amount of mildew (S^2) on the hops.

Ref. no. Z 4. 1918: Very mouldy (S^3) on the leaves. 1919: No observations made. 1920: On August 28 there was one spot of mildew on one leaf; no observations were made in October.

Cuttings of W 56, Y 28 and Z 4 taken in the winters of 1918–19–20 all proved persistently immune in the greenhouse.

In 1919 eleven cuttings of OR 38 in pots were stood[1] in the hop-garden on June 13. On October 23 when all were examined one cutting[2] was found to be infected very slightly, there being a tiny "powdery" patch on the undersurface of two leaves. In this season fourteen clone-plants of OR 38 [which were of the same age as the cutting (noted above) which became infected and which had been potted up in similar soil] had been tested in the greenhouse and had all remained persistently immune. With regard to the plants in pots in the open, no mildew was present on them at the end of September. By the date October 23 several light frosts had occurred; it appears, then, safe to infer that the immunity of OR 38 was broken down or partly broken down by certain climatic conditions, possibly low temperature. (The case of Z 25, noted above, also supports this view.)

Ref. no. OR 39. This seedling on being raised in the greenhouse from seed in 1914 proved, like OR 38, to be persistently immune. In the hop-garden it showed, in 1916, a trace of mildew, two lateral shoots from the main stem having one leaf each bearing a few mildew-patches. In 1917 one small mildew-patch occurred on the undersurface of one leaf of a lately-developed sideshoot. In 1918, 1919 and 1920 the plant remained free from mildew, although its neighbour OR 38 was infected

[1] The pots were stood on a board, to prevent any contact between the plant, or the soil in the pot, and the soil of the hop garden.

[2] This was a cutting of the clone-plant W 56.

to the extent of S^3, S^1 and S^2 in those years respectively. In the greenhouse, 35 cuttings of OR 39 have all proved persistently immune when tested in various seasons.

Ref. no. OY 18. No mildew was present in 1918; a trace of mildew occurred in 1919 and 1920. The behaviour of its neighbours on either side was as follows: OY 17 (♀), 1918, S^2; 1919, S^1; OY 19 (♀), 1918 and 1919, S^3.

Ref. no. DD 31. A trace of mildew occurred in 1919, which was the first season after the seedling had been planted out in the open. As a young non-flowering plant it remained persistently immune in the greenhouse for three consecutive seasons (1916–17–18). In 1920 no suitable material for infection was present in the hop-garden in the autumn.

Ref. no. HH 20. This seedling showed a trace of mildew in 1918. Its neighbour HH 19 (♀) was S^3 in 1918, and its other neighbour HH 21 (♂) was S^2. No suitable material was available in 1919, and the seedling was then grubbed up for want of room.

Ref. no. HH 44. A trace of mildew occurred on this seedling each season from 1917 to 1919; in 1920 no suitable material was available. Its neighbour HH 43 (♀) was S^2 in 1918 and 1919.

Ref. no. II 13. In 1918 a minute patch of mildew occurred on one leaf only. No suitable material was available in 1919 and the plant was then grubbed up for want of room.

Ref. no. II 24. No mildew occurred on this seedling in 1918 and 1919; its two neighbours, II 23 (♀) and II 25 (♀) were both S^2 in 1918.

Ref. no. II 30. In 1918 this seedling remained free from mildew, although a lateral shoot of II 31 (♂) which was virulently affected with mildew twined round it. No suitable material was available in 1919 and 1920.

Ref. no. 316. A minute trace of mildew occurred in 1919 and in 1920—in the latter season the small patches of mildew were confined to the undersurface of one leaf only.

Summarising the histories of the 27 seedlings which are immune in the greenhouse we find that 5 seedlings (Z 2, Z 17, Z 42, II 24, II 30) have remained immune in the hop-garden. All these are ♂ plants and as such have been less severely tested under the special conditions noted above (p. 149) than the ♀ seedlings; further, two of them (Z 17 and II 30) have obviously been insufficiently tested. Taking the evidence as a whole, one is led to the conclusion that in all probability these five seedlings need only some particular weather conditions, coupled with a certain stage of growth, to show in the open at least the S^1 stage of susceptibility.

It is interesting to find that the majority of the seedlings fall into the class in which susceptibility in the open varies from O to S^1. Of these seedlings six (V 92, V 93, Z 14, Z 31, OR 39, OY 18) are males, and five (V 91, Z 25, OA 49, OB 34, OD 19) are females. The injury caused by the mildew to plants which show a susceptibility of only S^1 grade would be negligible from the practical or commercial standpoint, so that these twelve seedlings could be classified as "commercially resistant[1]."

The six seedlings (OE 14, DD 31, HH 20, HH 44, II 13, 316) which so far have shown only S^1 in the open would similarly come into the "commercially resistant" class, if they can be considered sufficiently tested.

The two (female) seedlings (Z 20, Z 22) which have varied in susceptibility in different seasons from O to S^2, and the one (female) seedling (OA 34) which has varied from S^1 to S^2 have so far suffered so little injury from attacks of the mildew that they also can be included in the class of "commercially resistant," notwithstanding the fact that the grade of susceptibility may reach to S^2.

The (female) seedling OR 38 has varied in susceptibility from S^1 to S^3, and in the season of 1918 (and to a less extent in 1917 and 1920) the crop of hops was appreciably damaged. It is perhaps significant that this is the seedling which showed one season a local and temporary susceptibility in the greenhouse (see above, p. 154).

While, therefore, as OR 38 clearly shows, the possession of immunity under greenhouse conditions is not infallibly an indication of immunity, or even of "commercial resistance," in the open, it does appear that at any rate a majority of the seedlings which show immunity in the greenhouse possess, when grown in a hop-garden under commercial conditions, a sufficiently high degree of resistance to be classified at "commercially resistant."

In all, 301 cuttings, taken from most of the 27 seedlings enumerated above, have been rigorously tested in the greenhouse from 1917 to 1920, and all have been found to be immune. In no case has any seedling of the wild hop which has proved immune in the greenhouse in any one season shown susceptibility when cuttings from it have been tested in the greenhouse in other seasons. Cuttings have been taken from "immune" seedlings (*e.g.* Z 2, Z 14, Z 25, Z 42, OB 34) after they have been growing for five years in a fairly heavily manured hop-garden, and from

[1] To remove any possible misconception, it may be stated here that none of these seedlings of the wild hop have any direct commercial value.

one seedling (OR 38) after it has shown a high degree of susceptibility (S^3) in the hop-garden; in all cases the cuttings have shown the original persistent immunity. There is no indication so far that cultivation has had any effect whatever in changing or modifying the original inherent "constitutional" character of the seedling.

As will be seen from Table IV, tests have been made in various seasons with certain seedlings to prove the continuance of their susceptibility under greenhouse conditions. Not only seedlings which are mildewed to the degree S^3 in the open, but also seedlings, like OA 25, which in certain seasons show only a trace of mildew in the hop-garden, proved fully susceptible in the greenhouse.

If we contrast the excessive susceptibility under greenhouse conditions of the cuttings of the seedlings Z 24, Z 26, Z 41 and OD 18 with the absolute immunity of the cuttings of Z 25, Z 42, OD 19—remembering that all the cuttings were taken at the same time from the parent-plants which had been growing for five years in the hop-garden within a few feet of each other—we have the most convincing evidence of the permanence of different "constitutional" characters in seedlings of the wild hop.

(*b*) "*Semi-immunity*" *under greenhouse conditions.*

On reference to Table III, it will be seen that 7 seedlings (Z 15, Z 23, Z 38, OC 6, BB 5, OA 33, OD 17) have shown "semi-immunity" in the greenhouse. The behaviour of these seedlings in the hop-garden has been as follows:

Ref. no. Z 15. 1917, 1918, 1919: A medium attack of mildew (S^2) on the hops. In 1917 and 1918 its neighbour Z 16 was mildewed to the extent of S^3. In 1920 Z 15 showed on several of its youngest leaves the arrested or circumscribed development of the mildew-patches characteristic of "semi-immunity"; otherwise the plant was free from mildew.

Ref. no. Z 23. 1917: Mildewed to the extent of S^2 on the hops. 1918: No mildew present. 1919: A trace of mildew. 1920: No suitable material present. The neighbouring seedling Z 24 was so susceptible that each season it became mildewed to the highest degree, and in 1918 and 1920 the crop was destroyed by mildew.

Ref. no. Z 38. 1917, 1918: Mildewed to the extent of S^2 on the hops. 1919: No mildew present, although some of the lateral shoots of Z 38 twined round the stems of Z 39, which bore very mildewed hops. Z 39 has each season been attacked S^3, and in 1918 had its crop entirely destroyed by mildew.

Ref. no. OC 6. 1917, 1918: No mildew present, while OC 5 (♀) and OC 7 (♀) were both mildewed S^2 and S^1 in those years respectively. 1919: OC 6 showed a trace of mildew; while OC 5 showed no mildew, and OC 7 a trace of mildew. 1920: No mildew present on OC 6, but a few leaves showed signs of having repelled mildew attacks; OC 5 was mildewed S^2 (OC 7 had been grubbed up).

Ref. no. BB 5. 1918: A mere trace of mildew, while BB 4 (♂) and BB 6 (♂) were both mildewed S^2. 1919: No mildew present on BB 5 (BB 4 and BB 5 had been grubbed up). 1920: A trace of mildew.

Ref. no. OA 33. 1917, 1918: No mildew present, while OA 32 (♀) was mildewed S^3 in both seasons. 1919: Both OA 33 and OA 32 were mildewed S^2. 1920: A minute trace of mildew on OA 33 (OA 32 had been grubbed up).

Ref. no. OD 17. 1917, 1919: This seedling showed considerable resistance to mildew, producing a good crop of healthy cones, while its two neighbours OD 16 and OD 18, growing on either side and so close that lateral shoots intertwined, were so severely infected that practically every cone was diseased. 1918: A mere trace of mildew in the cones, while the crop of OD 16 and OD 18 was destroyed by mildew.

Summarising the histories of the above seedlings, we find that two seedlings, OC 6 (♂) and BB 5 (♀) have varied in susceptibility in the hop-

Table V.

Analysis of 480 *seedlings of the wild hop.*

Degree of susceptibility	No. of seedlings	%
S^3 (including S^3†) *each year* (11 ♂; 85 ♀)	96	20·00
S^2 to S^3 (sometimes S^3†) (35 ♂; 80 ♀)	115	23·96
S^2 (54 ♂; 26 ♀)	80	16·67
S^1 to S^2 (41 ♂; 48 ♀)	89	18·54
S^1 (19 ♂; 12 ♀)	31	6·46
O—S^1 (9 ♂; 9 ♀)	18	3·75
O (all ♂)	6	1·25
O—S^2 (9 ♂; 18 ♀)	27	5·63
O—S^3 (all ♀)	3	·60
S^1 to S^3 (5 ♂ ; 10 ♀)	15	3·12
	480	100

garden from O to S^1, and that one seedling OD 17 (♀) has shown the grade S^1 consistently. These three seedlings may apparently be classed as "commercially resistant" in the open.

All the other seedlings are liable in certain seasons to be mildewed to the extent of S^2.

As already mentioned, 480 seedlings have been planted out in the Experimental Hop-garden. In October of each year the susceptibility of each seedling has been noted. For the greater number of these seedlings, the records have been kept for four years (1917 to 1920). An analysis of these records gives us the following classification on p. 159.

Of the 480 seedlings, 189 (or 39·38 per cent.) proved to be males, and 291 (or 60·62 per cent.) proved to be females.

A separate analysis of the records of the two sexes gives us the following classification:

Table VI.

Analysis of 189 ♂ *seedlings of the "wild hop."*

Degree of susceptibility	No. of seedlings	%
S^3	11	5·82
S^2—S^3	35	18·52
S^2	54	28·57
S^1—S^2	41	21·69
S^1	19	10·05
O—S^1	9	4·76
O	6	3·17
O—S^2	9	4·76
S^1—S^3	5	2·65
	189	100

Table VII.

Analysis of 291 ♀ *seedlings of the "wild hop."*

Degree of susceptibility	No. of seedlings	%
S^3 (including S^3†)	85	29·21
S^2—S^3 (sometimes S^3†)	80	27·49
S^2	26	8·93
S^1 to S^2	48	16·49
S^1	12	4·12
O—S^1	9	3·09
O—S^2	18	6·19
O—S^3*	3	1·03
S^1—S^3*	10	3·44
	291	100

* See remarks below at p. 162.

The percentage figures in the two tables given above show a higher grade of susceptibility among the ♀ seedlings than among the ♂ seedlings. It would be unsafe, however, to consider this proved, owing to the circumstance (see above, p. 149) that the observations as to the incidence of the mildew were made at a time peculiarly favourable for attacks on the ♀ plant. The ♂ and the ♀ plants would need to be tested at a time when each provided the same amount of infectible material before any inference could safely be drawn as to their relative susceptibility. Of the 27 seedlings showing immunity in the greenhouse, 14 are ♀ and 13 are ♂.

Since excellent material to test the degree of susceptibility was always present each year in the case of the ♀ plant, it will be safer to use the figures in Table VII rather than in Table VI on which to base an estimate of the percentage of the seedlings in various grades of susceptibility.

In the highest grade of susceptibility in which the plant was *each season* mildewed to the extent of S^3, there are 85 seedlings. Of these, no less than 34 had their crop of hops entirely destroyed in one or more seasons[1].

In the next class, the incidence of mildew varied from S^2 to S^3 in different seasons. This class comprises 80 seedlings. Of these, 21 seedlings had their crop of hops destroyed by mildew in some season[2]. If we add together 34 and 21, we get 55 as the number of seedlings which have had the entire crop of hops destroyed in some season or seasons. This number represents 18·90 per cent. of the total number of the ♀ seedlings.

Referring back to Table VII, we may consider the first class of 85 seedlings showing S^3 each season (which represents 29·21 per cent. of the ♀ seedlings) as exhibiting excessive susceptibility to mildew, probably greater than that shown by any commercial variety of hop cultivated to-day.

To the class of very susceptible seedlings may safely be added the 80 seedlings of the S^2 to S^3 class. This gives us 165 seedlings (or 56·70 per cent. of the total number) belonging to a grade of susceptibility in which virulent attacks of mildew are common.

[1] The actual records for these 34 seedlings are as follows, where the integer gives the number of seedlings, and the numerator of the fraction the number of times the entire crop was destroyed and the denominator the number of seasons during which observations were made: $2\frac{3}{4}$, $5\frac{2}{4}$, $7\frac{1}{4}$, $2\frac{2}{3}$, $7\frac{1}{3}$, $11\frac{1}{2}$.

[2] In all cases $S^{3}\dagger$ was reached only once by these seedlings, the actual records being as follows: $1\frac{1}{5}$, $9\frac{1}{4}$, $6\frac{1}{3}$, $5\frac{1}{2}$.

In the class S^2, consisting of seedlings which have shown a medium attack of mildew each season, we have 26 plants, or 8·93 per cent.

In the next class, where the incidence of mildew varies in different seasons from S^1 to S^2—that is, from a trace of mildew to an attack of medium intensity—we have 48 plants, or 16·49 per cent. In this class an appreciable degree of resistance to the mildew begins to be shown by some of the seedlings, but it is in the succeeding classes that this phenomenon is most clearly seen. One seedling, however, in the present class proved to be commercially resistant.

In the class S^1, *i.e.* those seedlings which have shown only a trace of mildew each season, we have 12 plants. Of these, 5 cannot be considered to have been sufficiently tested, leaving us with 7 seedlings which have proved to be persistently resistant in the open. Among these 7 seedlings, there are 4 which are immune, and 1 semi-immune, under greenhouse conditions.

In the class O–S^1 we have 9 seedlings, of which 1 has been insufficiently tested. These 8 seedlings are "commercially resistant" in the open; 5 of them are immune, and 1 semi-immune, under greenhouse conditions.

In the class O–S^2, in which there are 18 seedlings, 2 plants (both of which show immunity in the greenhouse) have proved to be "commercially resistant" in the open. The other seedlings in this class, while certainly showing resistance to the mildew, are liable to fairly serious attacks in some seasons, and therefore cannot be considered as "commercially resistant." Among these are 2 seedlings which are immune, and 1 which is semi-immune, under greenhouse conditions.

The 13 seedlings in the classes O to S^3 and S^1 to S^3 do not constitute in either case a proper group. Of the 3 seedlings showing O to S^3, one only possesses probably a slight degree of resistance. Of the 10 seedlings showing S^1 to S^3 in different seasons, 9 almost certainly belong to the S^2 or S^3 class, while the remaining plant is OR 38 (immune in the greenhouse), which is fully discussed above (p. 154).

As constituting the group, then, of "commercially resistant" plants, we have the following 18 seedlings (representing 6·19 per cent. of the 291 ♀ seedlings), distributed in the following classes:

S^1 to S^2: OA 34.

O to S^2: Z 20, Z 22.

S^1: OC 25, OD 17, OE 14, FF 9, HH 20, HH 44, II 13.

O to S^1: V 91, Z 25, OA 49, OB 34, OD 19, OY 24, BB 5, HH 9.

SUMMARY.

1. The "wild hop" (*Humulus Lupulus* L.) is composed of a number of forms[1] which show distinctive physiological, or "constitutional," characters, as measured by the grade of susceptibility to the attack of the mildew *Sphaerotheca Humuli*. These characters vary from extreme susceptibility, shown both in the open and under greenhouse conditions, to a high degree of resistance in the open and complete immunity in the greenhouse. Of 291 ♀ seedlings, 165 seedlings, or 56·70 per cent., show extreme susceptibility, while 18 seedlings, or 6·19 per cent., are "commercially resistant" in the open. The remaining seedlings fall into groups representing intermediate grades of susceptibility.

2. Of 480 seedlings, ♂ and ♀, 27 seedlings, or 5·63 per cent., are completely immune—and 7 seedlings, or 1·46 per cent., semi-immune—under greenhouse conditions.

3. The majority of the seedlings which show complete immunity under greenhouse conditions show a high degree of resistance in the open.

4. The distinctive degree of susceptibility possessed by a seedling, both as shown in the open and under greenhouse conditions, has shown no change after the plant has been cultivated for five years in a manured hop-garden.

[1] The question as to whether these forms possess distinctive morphological characters is reserved for another occasion.

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ON THE FLEECES OF CERTAIN PRIMITIVE SPECIES OF SHEEP[1]

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(With Plates I and II.)

INTRODUCTION.

At the present time extensive breeding experiments between Blackfaced ewes and Southdown rams are in progress with the ultimate object of improving the fleece of the Scottish hill-sheep. To this end precise knowledge of hair and of wool is necessary.

In the fleece of the Southdown there is but one kind of fibre, recognised as wool, while in that of the Blackfaced there are fibres very distinct one from the other and recognised as hair and wool respectively, and also other fibres appearing to be of intermediate character, the exact nature of which is not known. These indefinite fibres render the above experiment extremely complicated yet full of interest to the student of genetics.

They may possibly be the common ancestral type of fibre from which both hair and wool have sprung, or they may be but degenerate forms of hair or wool or of both.

An investigation of the characters, functions, and natural variability of the fleece of the wild and primitive species of sheep, some of which are known to have played a part in the production of the modern domesticated breeds, may throw considerable light upon this problem of wool-improvement and will provide standards to which the component fibres of the modern fleeces may be referred.

One of the distinguishing characters of mammals is the presence of hair. Classed according to the nature of its growth, it is of two kinds: temporary, being shed usually once a year and replaced by new hair, and permanent, having a perennial growth. The summer coat is not shed, it develops further to become the winter hair. The winter hair is shed in the spring, the new summer coat replacing it.

[1] A grant in aid of publication has been received for this communication.

But classed according to the length and structure of the hair there are two types also. One is relatively short and curled and is hidden by the longer, coarser, straighter kind. These two types of hair, because of the difference in their length and disposition, form two coats: an inner one of fine, curly fibres, forming an open-meshed mat snugly applied to the surface of the skin, and an outer one of stout, up-standing fibres, projecting well beyond the inner coat.

The two coats appear to serve different functions and this is associated with very distinct differences in structure. The outer one shields its wearer from excessive heat and cold, from wet, and from injurious contact with objects generally. It is cold- and heat-excluding, the general direction of its component fibres is such that it forms an efficient downward watershed, draining off the rain as it falls; its fibres present a surface upon which snow does not readily find a lodgment; and it protects the curly, delicate, fine hair of the inner coat from entanglement in thorny thickets.

The inner coat is heat-retaining in function, for in its meshes air is held. The inner coat is the wool of commerce. Many other mammals also possess this coat and it is a matter of conjecture why man chose the sheep as the source of his supply.

I am indebted to Professor J. Cossar Ewart, F.R.S., of the University of Edinburgh for the samples with which this study was made, and to Major H. J. W. Bliss of the British Research Association for the Woollen and Worsted Industries for much helpful criticism. A grant from the Carnegie Trust has enabled me to illustrate this paper with appropriate plates.

THE FLEECE OF *OVIS AMMON POLI.*

Marco Polo's "exceeding great wild sheep, having horns, some of them six spans long," are found on the mountain ranges of the Pamirs —"the roof of the world." They have been shot 18,000 feet above sea-level. The climate of this region is very rigorous, with long winters, rain and snow and bitter winds, but the water-courses and valleys are rich in mountain grasses, and even on the high passes in March snow is rarely so deep as to prevent the herds of the Kirghiz from finding pasturage.

Two samples from the summer and winter coats respectively were available for examination, and both were taken from the mid-dorsal line between the shoulders.

The summer coat (Pl. I, Fig. 1, left) on inspection showed one kind of fibre having the general appearance of hair as opposed to wool. But classed according to length and colour, there were three groups:

1. Numerous fibres uniformly 3 cms. in length and having shafts of uniform calibre. The free end of each was broken and each bore along its length the marks of numerous transverse fractures. In colour they were white in the proximal third and more or less pigmented a ruddy brown in their distal two-thirds.

2. Others, less numerous, were shorter, ending in unbroken, long, tapering tips and uniformly pigmented.

3. Complete, deeply and entirely pigmented, very small fibres, ranging from 1–7 mms. in length, forming a bristly mat upon the surface of the skin.

The winter coat (Pl. I, Fig. 1, right) was of two kinds, an outer one of hair similar to the longest hairs of the summer coat, and an inner coat of fine curled wool.

The great majority of the hairs were 5 cms. in length, some very slightly coloured and others entirely devoid of pigment. Not one possessed its tip and many had been broken off nearer the skin. No smaller or intermediate hairs were present.

The wool fibres when stretched to their fullest extent averaged 3·5 cms. in length, but there was considerable variation. The inner coat of wool formed a mat among the bases of the hairs 2 cms. thick.

The following measurements were made by Professor Barker of Leeds University for Professor Ewart.

	Hair					Wool		
				Diameter of				
Sample	Diam.	Length	Waves per inch	Tip	Root	Diam.	Length	Waves per inch
Ovis ammon poli								
Summer coat	·009	1·1	3	·002	·01	—	—	—
Winter coat	·0103	1·6	5	·005	—	·001	·8	8
O. ammon hodgsoni	·0107	1·0	6	·00125	·0107	·001	1·03	20
O. ammon ammon	·006	1·28	8	·00105	·0071	·00075	·93	20

This table shows that there is no wool in the summer coat of Marco Polo's sheep. In the case of the other species only the winter coat was available for examination.

The Hair (Pl. I, Figs. 2*a*, 3, Pl. II, Figs. 4, 5). A transverse section of a typical hair from either the summer or the winter coat showed the following structure. A central medulla consisted of shrunken cells and cell-elements lying amid irregular spaces. Surrounding the medulla was the cortex, composed of several concentric layers of flattened cells. External to the cortex, the hyaline colourless scales of the cuticle were sought but though scores of hairs were carefully examined, after different methods of preparation

and with different methods of illumination, these scales, with their free edges typically directed towards the tip of the hair, could not be found until the heavily pigmented very small hairs from the summer coat were examined.

In the longer and older hairs the cuticle had disappeared (Pl. II, Fig. 4), probably by attrition during life, leaving the cortical cells exposed, and only near the bases of such hairs could remnants of the cuticle be found. In the case of the shorter and younger hairs the cuticle had been preserved since these hairs had been amply protected by the longer ones (Pl. II, Fig. 5).

The hair fibres were never entangled, and naturally do not felt as they are too straight, too stout, and too up-standing, and in the case of the young hairs upon which the cuticle is still present, the cuticular scales have free edges which are not serrated.

Figs. 2 and 3 show that the disposition of the pigment granules of a hair is such that they outline the cells of the cortex. It was found that when a winter white hair was mounted in weak balsam it assumed the coloration of a pigmented summer hair. This was possibly due to the withdrawal of air as the following experiment shows. A white hair, mounted in weak balsam and examined under the microscope, was subjected to gentle pressure along its length by means of a pencil-point. The hair ruptured, and air-bubbles rushed out, leaving the fibre pigmented. On removing the pressure the air rushed back and the hair became once more white.

The medulla of a hair could not be seen through the cortex, but it seemed that the spaces of the medulla were occupied by air which made its way among the cortical cells. If air comes to lie between the pigment of the cortex and the eye of an observer, the refractive properties of the air-film render the pigment invisible. The presence of air within a hair is associated with age: a white hair is an old hair. The air-film, moreover, gives a heat-retaining property to the outer coat and incidentally provides a protective coloration. It was found that the air-film prevented aqueous dyes from entering the fibres. It is suggested that kemp is such a fibre, a hair prematurely old.

The Wool. The wool fibre has the same essential histological structure as a hair save that it is finer—0·1 of the diameter of a hair—non-pigmented, and has many more waves to the inch. It is so constructed that it is impossible to distinguish between cuticles and cortex. One cell with free edges serrated and projecting towards the tip, occupies

the whole circumference of the fibre in striking contrast to the surface structure of a hair (Pl. II, Fig. 6 *a*, *b*, *c*).

In the majority of wool-fibres no medulla could be identified containing air-spaces, but in a few definite air-filled central areas were seen (Pl. II, Fig. 6 *d*).

The Fleeces of Other Species of Sheep.

For purpose of comparison the hair and wool of *O. ammon hodgsoni*, *O. ammon ammon*, *O. vignei*, *O. montana*, and *O. orientalis* were examined. Save for certain minor unimportant differences, all were identical in structure and did not differ from the hair and wool of *O. ammon poli*.

In the case of *O. orientalis*, however, the character of the inner coat was remarkable in that instead of the fibres forming a much entangled mat (Pl. II, Fig. 7 *b*) as in the case of the other fleeces, they formed natural locks very similar in form to those of the wool of the modern breeds (Pl. II, Fig. 7 *a*). I am indebted to Professor Ewart for calling my attention to this interesting and perhaps important fact. Of all the primitive wools this most resembles in disposition the wool of commerce of to-day.

The two kinds of fibres in the fleeces of the primitive species of sheep were absolutely distinct and no sort or grade of intermediate fibre was found. Careful search for a fibre of intermediate character was made but without success, each fibre was either hair or wool, definitely and unmistakably. Thus the fibres of intermediate character found in certain modern fleeces cannot be regarded as transitional forms and the question as to whether hair and wool are different in their origin and development, or whether they result from the divergent development of a common type of fibre of intermediate character cannot be answered. The exact relation of hair and wool must be sought in other mammals.

The hair and wool of the primitive species of sheep may be accepted as the typical examples of these fibres and as standards to which the fibres in the modern fleeces may be referred.

SUMMARY.

1. The fleeces of certain wild and primitive species of sheep were examined.

2. That of Marco Polo's sheep was found to be the one most suitable for examination, since samples of both summer and winter coats were available. In the former coat there was hair only and still growing. In the latter coat it was mostly full-grown and much less pigmented. The

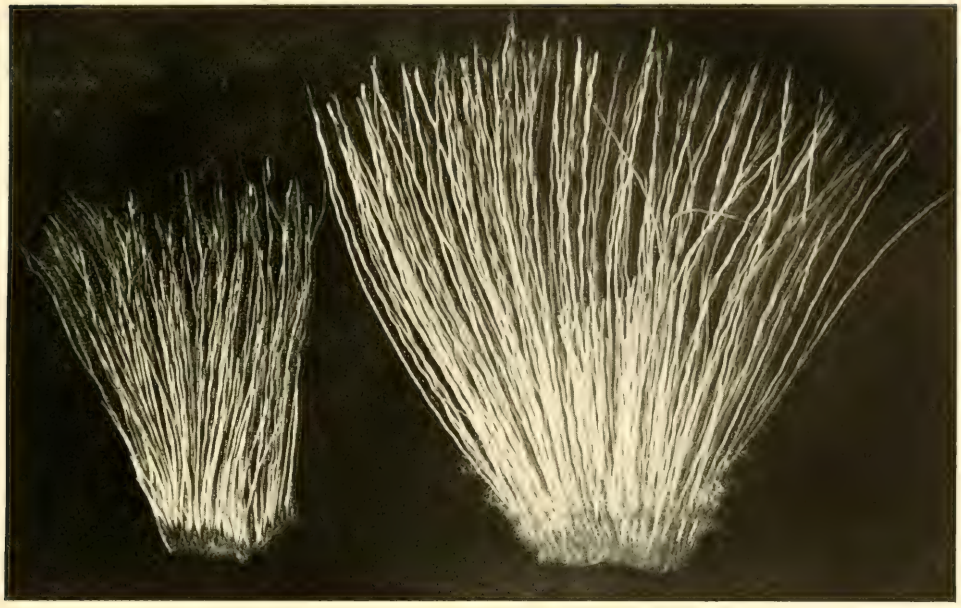

Fig. 1.

Fig. 2 *a*.

b.

c.

Fig. 3.

white appearance was due to the presence of a film of air between the pigment granules of the cortex and the eye of the observer.

3. Wool is present in the winter coat but absent from the summer one.

4. The microscopical structure of hair and of wool are briefly described and attention is called to the disposition of the wool in the fleece of *O. orientalis*, since this most closely resembles that of the wool of the modern domesticated breeds.

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EXPLANATION OF PLATES I AND II

Fig. 1. *O. ammon poli*: Summer (left) and winter (right) fleeces. $\times 1\frac{1}{2}$. In the summer coat the distal two-thirds of the longest hairs are pigmented; young heavily pigmented hairs form a dark mat upon the skin: there is no wool.
In the winter coat the hair is longer and the tips are broken; there is much less pigment; there are no young hairs; and there is an inner coat of wool.

Fig. 2. The cortical cells of hair. $\times 110$. (*a*) The hair of *O. ammon poli*; (*b*) of *O. ammon hodgsoni*; (*c*) of *O. ammon ammon*. The pigment is arranged around the cells.

Fig. 3. The cortical cells of the mature hair of *O. ammon poli*. $\times 450$. The cells are those of the cortex; there is no overlapping and the cells are arranged in the form of a mosaic.

Fig. 4. The lateral edge of a full-grown hair, $\times 420$, to show that there is no cuticle.

Fig. 5. A young hair from the summer coat, $\times 1230$, to show the presence of the cuticular scales.

Fig. 6. Wool. $\times 600$. (*a*) *O. ammon poli*. (*b*) *O. ammon ammon*. (*c*) *O. ammon hodgsoni*. (*d*) A fibre showing the presence of air-spaces within the medulla.

Fig. 7. Wool. $\times 3$. To show the disposition of the fibres. (*a*) *O. orientalis*. (*b*) *O. ammon poli*.

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OBSERVATIONS ON THE INSECTS OF GRASSES AND THEIR RELATION TO CULTIVATED CROPS

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I. INTRODUCTION.

The insects of grasses may be divided into two distinct groups, those which actually feed on the grasses and those which shelter among grass during the winter period. For the purpose of this paper particular emphasis will be laid on such insects as, while using grasses for shelter or food, finally migrate to cultivated crops for the completion of their life-history, for the development of later broods, or, as in the case of sheltering insects, for food when the crop is at the critical stage which satisfies their requirements.

That insect pests of cereals use grasses as intermediate hosts is a well-established fact (see Theobald(18), Miall(11), Washburn(19) and Lugger(10)). So far, however, little attempt has been made to establish definitely the part played by grasses in harbouring the pests, and we know very little of the degree of infestation of those which grow about our hedgerows and waste places. Accurate knowledge under this head would be of value to the agriculturist in controlling insect pests by the more efficient upkeep of hedges and ditches.

This subject for investigation was suggested by Dr A. D. Imms, M.A., to whom I am grateful. My thanks must also be expressed to Mr A. Roebuck, Head of the Department of Agricultural Biology at Harper Adams College, for information, suggestions and constant advice. The observations were taken over the period from mid-October 1919 until the end of June 1920.

The winter period was very mild and undoubtedly favoured the persistence of growth of plants and the continued feeding of many insects. The spring and early summer, however, was wet and cold with short periods of bright warm days, which state was so unfavourable that it was not until May 18th that the Frit Fly adults were observed on the oat crop.

II. METHODS.

In order to arrive at some definite conclusion as to what insects are found feeding and wintering in grasses, examples of the various species dug up from headlands, hedgerows, waste ground and arable fields, were taken into the laboratory and examined, each shoot being cut open for careful scrutiny. The date, locality, species of host plant, and the number and variety of insect stages present were recorded for further consideration. Of the insects found some were preserved in spirit, others kept in captivity, if larvae or pupae, for examination in subsequent stages, while many were dissected for microscopic examination.

While the laboratory work was proceeding the crops growing on the College Farm were kept under observation and, where they were attacked by any insects of the species found feeding amongst grasses, data as to cultivations, date of sowing and infestation were secured. In this connexion particulars are included of the attack by Frit Fly on winter wheat in the early part of 1920.

Special note was taken of insects found sheltering in the plants examined, since definite information concerning the means whereby many insects pass the winter is lacking.

With regard to root-feeders such as "wireworms" and "Leather-jackets" the difficulty of taking them actually feeding presented itself, but where symptoms of attack in grasses and corn crops were identical it was considered justifiable to draw conclusions.

The foregoing may be considered as the winter methods, those obtaining in the summer period varying in that less time was spent in the dissection of grasses and more in observing the attacks of leaf-feeding and leaf-mining insects under field conditions. Here again arose the difficulty of the root-feeders and night-feeders, so that evidence of the larvae having eaten and their proximity to the attacked plant when taken were noted and inferences drawn.

III. SOIL AND LOCALITY.

Most of the observations were made on the College Farm, where there are two main types of soil, viz. the heavier loams merging into clays and the lighter loams emerging into soils of sandy texture. Geologically these types are represented by the Lower Red and Mottled Sandstone and the Pebble Beds, both of the New Red Sandstone formation. The locality is approximately 220 feet above sea level and is exceptionally free from smoke of factory, foundry or town.

The rainfall is normally about 24·5 inches annually, falling chiefly in winter and spring.

The locality is well sheltered on all sides by belts and clumps of trees, chiefly elm, lime, ash and oak, and these, with the woods of the neighbourhood attract birds which, in their foraging doubtless destroy numbers of insects harmful alike to grass and arable land.

On the newly broken up grassland it was noticeable that pigeons, rooks, jackdaws, plovers and starlings spent most of their time, in preference to the older arable fields the insect life of which had attained its normal level.

Grasses grow very luxuriantly here so that there is abundance for such insects as infest them, hence the effect of insect migration is felt continuously.

IV. HARVEST TO SPRING SOWING—WINTER PERIOD.

Table of Insects Feeding and Host Plants.

	Host
1. *Coleoptera:*	
Agriotes obscurus, L.	Arrhenatherum avenaceum
	Dactylis glomerata
	Lolium italicum
Athous haemorrhoidalis, F.	Cynosurus cristatus
	Lolium perenne
Melolontha vulgaris	Agrostis stolonifera
	Poa pratensis
	P. trivialis
2 *Hymenoptera:*	
(Unidentified)	Galls on Agropyrum repens
3. *Lepidoptera:*	
Apamea secalis, Bjerk.	Dactylis glomerata
	Agrostis stolonifera
	Lolium perenne
	Poa pratensis
	Trisetum flavescens
Triphaena pronuba, L.	T. flavescens
	Poa pratensis
	Lolium perenne
Agrotis exclamationis, L.	L. perenne
	L. italicum
4. *Diptera:*	
Cecidomyia destructor, Say.	Agropyrum repens
	Dactylis glomerata
Cecidomyia spp.	Agropyrum repens
	Dactylis glomerata
	Lolium perenne

Oscinis frit, Linn. . . .	Trisetum flavescens
	Arrhenatherum avenaceum
	Agrostis stolonifera
	Holcus lanatus
	Lolium perenne
Pachyrrhina imperialis, Mg. .	L. perenne
	L. italicum
	Arrhenatherum avenaceum
	Holcus lanatus
	Bromus sterilis
Tipula oleracea, L.	Lolium italicum
	Arrhenatherum avenaceum
	Agrostis stolonifera
	Poa pratensis
Sciara spp.	Trisetum flavescens
5. *Thysanoptera:*	
Limothrips cerealum, Hal. .	Dactylis glomerata
	Holcus lanatus
	Lolium perenne
6. *Hemiptera:*	
Macrosiphum granarium (Kirb.)	Arrhenatherum avenaceum
7. *Collembola:*	
Sminthurus luteus (Lubbock) .	Lolium perenne
Orchesella cincta (Linn.) . .	L. perenne

Of the insects feeding in winter, we find among the Coleoptera the larvae of the click beetles. "Wireworms" as defined by Roberts (14) are "The larvae of the genus Agriotes and that of *Athous haemorrhoidalis*." Their distribution is somewhat localised for Ford (7) says that in Cheshire he found the common wireworm as the larva of *Agriotes obscurus*. A similar state of affairs obtains in Shropshire where *Agriotes obscurus* predominates, *Athous haemorrhoidalis* coming second in order of importance. In this district the wireworms were found in greatest numbers on sandy soils having abundance of humic matter in the form of root fibres. Land recently broken up was badly infested and calculations showed in several fields numbers varying from 26,780 to 331,700 wireworms per acre, the former case existing after a crop of linseed. The larvae of *Athous haemorrhoidalis* apparently prefer pastures and meadows to arable land; only two specimens were taken in arable land and then on land which had been broken up in 1918. They were fairly numerous along hedgesides where they attained great size, feeding amongst the roots of grasses and hedge plants. Some difference of opinion still prevails as to the mean depth of wireworms in the soil. During the winter careful examination of nine fields, seven of which were arable, showed that the majority of larvae were located from 1 inch to 4 inches below

the surface, except in the case of an attack of spring oats in April and May when they were found in the top inch of soil, many actually crawling out of the soil to attack the stems of young plants above the soil line. Numerous cases of larvae boring upwards in the stem were observed, the most persistent larvae being those of *Agriotes obscurus* from 8–12 mm. in length; in conjunction with this attack the various Tipulid larvae were also doing considerable damage.

The characters of Agriotes larvae as given by Roberts(14) enabled these wireworms to be readily distinguished. Roebuck in the 1918 Report of the Intelligence Department, Plant Disease Branch, states that "a downward movement of these larvae to protect themselves from cold was not apparent." This has been verified, the wireworms being taken within 3 inches of the surface on frosty mornings, and, even after continued frost, no migration to lower depths was observed.

The larvae of *Melolontha vulgaris* were found chiefly in meadows and waste land, feeding on any available decaying herbage. They showed a distinct preference for lighter soils and were consistently taken in heaps of road scrapings which had been left along roadsides. Here the larvae thrived, the compost of soil, grit, manure and decaying vegetation being evidently most favourable for them. No specific attack was noticed in the locality, though the larvae are reported as attacking mangels in Carnarvonshire in 1917 and potatoes in Norfolk, Surrey and Cardiff in 1918, those in Surrey being grown on an old lawn which had been broken up during the winter(1).

Lema melanopa and *Sitones lineatus* were frequently found sheltering, the former head foremost in the hollowed portion of the stems of tall oat and cocksfoot grasses, and the latter low down amongst the dry dead leaves of cocksfoot, particularly in the vicinity of a field of "seeds." The attack of *Sitones* spp. on leguminous crops is very universal and on a field of beans 32 weevils were collected either feeding or resting from 62 plants, this was about 11 o'clock on a mid-April morning. Curtis(6) alluding to this ubiquitous weevil in 1844 states, "I well remember that in April and May I could not find a pea-field where the lower leaves of some plants were not eroded, the beans were equally marked."

The Hymenoptera are represented by only one species which formed galls on couch grass. This insect, which has not been identified, was found only during the winter and spring and I have found no instance of its being taken on any other grass or on any cereal crop.

The most predominant Lepidopteron was *Apamea secalis*. The larvae were found feeding internally in the base of the shoots of both grasses

and cereals from October till the beginning of June. The smaller, and therefore younger, larvae work upwards and the older ones downwards in the stem, the latter case being probably after migration from one plant to another, or from one shoot to another, and just prior to pupation. In only one case was a larva found externally and that on a stem of Golden Oat grass, possibly it was migrating or seeking a site for pupation —it eventually died after two days in captivity.

In February, Apamea larvae were found in conjunction with frit larvae attacking winter wheat ("Iron" variety) taken after seeds.

In 1918 similar attacks are recorded on wheat and oats in Shropshire, Hampshire and Wiltshire—being especially noted on ploughed up pasture. One is inclined to the belief that the larvae of *Apamea secalis* feed indiscriminately on grasses or cereals in almost any situation, be it pasture, meadow, headland, hedgerow, waste land, roadside, or grasses on bare patches in arable fields. An examination on May 31st of self-sown wheat and oats growing amongst clover and beans showed that of 28 tillers of wheat four were attacked, while in oats only one out of 24 suffered from Apamea.

The larvae of the *Agrotis* spp., specimens of which were taken feeding on the roots of perennial rye grass, were reported as attacking the young plants of wheat and oats in Staffordshire and Shropshire in 1918.

Triphaena larvae were taken feeding on the roots of golden oat grass; they are, however, general in their feeding habits and often go to rye grasses and meadow grasses.

The larvae of *Odonestis potatoria* which feeds on various grasses was taken on cocksfoot but was not observed to attack any cereal crop. One *Tortrix* spp. was taken feeding on oats and on the tall oat grass: it was small, about 8–10 mm. varying in colour from sooty to grey, sparsely hairy and exceedingly active. It fed both internally and externally. So far, attempts at rearing it have proved unsuccessful.

Among the Diptera, the Hessian Fly was found exclusively on Cocksfoot, as many as five pupae being taken from a single node. A specimen was also found on couch grass. Examination of cocksfoot grasses in autumn and early winter showed large numbers of empty pupal cases from which the autumn brood had hatched, also many pupae, which were kept and from which adults emerged in May. Records of attack by this fly are as follows: (1917) "Attacks by this fly on wheat were recorded from Durham, Lancashire (where it was abundant on a crop of late sown wheat near Birkenhead), Shropshire and Oxfordshire (fields of 'Sensation' wheat destroyed)"..."and in the Eastern Counties."

In 1918 a "slight attack in combination with *Oscinis frit*" is recorded(1) at Ormskirk, while at Harper Adams Agricultural College there was an attack on "Square Heads Success" wheat. Theobald mentions the second brood as attacking couch and timothy grasses, so far, however, I have not taken it on timothy. Allied to the foregoing are species of Cecidomyia which were found in great numbers on decaying vegetation during the winter. The "red maggots" accompanied "frit" and "Apamea" in their attacks on wheat and grasses. Larvae were taken on couch, cocksfoot, golden oat and perennial rye grass—the "red maggots" so characteristic are perhaps more saprophytic than parasitic in habit, especially is this so with cocksfoot grass.

The Frit Fly, *Oscinis frit*, was found first on November 19th when twelve larvae were taken from one plant of *Arrhenatherum avenaceum* —the tall oat grass. The plant was growing in the hedgerow bordering a field in which oats were badly attacked previously, so evidently the autumn brood had been egg-laying in the neighbouring grasses. Later, larvae were found feeding in perennial rye grass, golden oat, *Holcus lanatus* and *Agrostis Stolonifera*. In early February an attack, previously mentioned, on wheat after "seeds" was noted in a field on the College Farm and on examination the following infestation was calculated: Plants "Fritted," 27 per cent; plants attacked by *Apamea* spp., 1·05 per cent. In several cases two "frit" larvae were found feeding in the same stem. Regarding burying "frit" and their migration, eight "frit" larvae *in situ* in wheat plants were buried at a depth of 4 inches in a 9-inch pot of medium loam and above were planted four healthy wheat plants and four oat seedlings with the second leaves showing. Though the pot was kept under constant observation none of the plants were attacked but grew vigorously and healthily and no perfect insects were observed to emerge. Collin(5), however, notes emergences from larvae buried from 7 inches to 9 inches deep.

Of root-feeders there were noted *Pachyrrhina imperialis* Mg., *Tipula oleracea* and the larvae of a Sciarid. The first-named feeds on plants in general, in the more moist parts of the field, preferring the rank growth near hedges, though it was found feeding amongst the roots of italian rye grass growing amongst beans. The larvae of *Tipula oleracea*, the common "Leather Jackets," were very destructive to barley seedlings in spring on newly broken-up grass fields of a moist character the soil of which was sandy and humic overlying clay. They are universal feeders and Roebuck(4) calculated in three different grass-fields 220,000, 13,000 and 120,000 larvae per acre.

Cameron (2) records the larvae of a species of Sciarid occurring "in small masses at the roots of grasses on which they were in all likelihood feeding."

Of the Hemiptera one species alone was observed, viz. *Macrosiphum granarium*. In 1917 "This aphis was reported as very abundant and causing considerable damage in the Eastern Counties, Oxforshire, Shropshire and Staffordshire on wheat and oats in July and August (1)." This insect was found on tall oat grass, singly in early autumn, and again in the summer period. Attacks on oats were simultaneous with attacks on hedgerow grasses.

Limothrips cerealum (Hal.) was the only Thysanopteron taken. It feeds through the winter, in the larval stage in hollow-stemmed grasses. In 1917 "A distinct case of considerable damage to "Giant Eliza" oats at Newmarket (Cambs.)" was recorded (1)—though Thrip attack is fairly general but often overlooked.

Two species only of the Apterygota were noted—*Sminthurus luteus* and *Orchesella cincta*. These developed in great numbers on a plant of perennial rye grass obtained from a damp waste place and kept indoors. No definite attack was noticed. Cameron states, with reference to the Apterygota as a whole, "As a rule they do not penetrate very deeply into the soil generally being found in the first three inches. In many cases where members of this order have been reported as doing damage to root crops, I doubt very much whether they are the direct agents of injury. The chances are that they are merely what we term 'followers of decay' accentuating the evil that has been caused by other pests." In 1914 Roebuck, referring to damage to wheat and the presence of *Isotoma palustris*, writes (16): "These sheltered in tiny holes in the ground and were never caught on the wheat, and, therefore, may have caused none of the damage." Subsequently, however, he tells me thay were abundant on wheat but were never seen actually feeding.

V. SPRING SOWING TO HARVEST—SUMMER PERIOD.

In addition to practically all those mentioned under "Winter Period" the following insects were observed feeding or sheltering in grasses.

Coleoptera:	
Rhizotrogus solstitialis	Grass roots in meadows
	Dactylis glomerata
	Lolium italicum
Lema melanopa, L.	Agropyrum repens
	Arrhenatherum avenaceum
Sitones lineatus	Amongst grasses in hedges

Hymenoptera:	
Pachynematus clitellus, Dolerus haematodes	Poa trivialis, Arrhenatherum avenaceum
Lepidoptera:	
Odonestis potatoria	Dactylis glomerata
Hepialus humuli	Lolium italicum
Arctia caia	Dactylis glomerata
Diptera:	
Agromyza nigripes	Agropyrum repens
Rhyphnus fenestralis	Lolium talicum

During the summer period attacks on both grasses and Cereals were noticed by wireworms, leather jackets, surface larvae, weevils and apions. Since most of these insects have been dealt with under the previous section only those not mentioned there will here receive consideration.

Rhizotrogus solstitialis is a general feeder in the larval stage and like other chafer grubs does damage on broken-up grasslands and to the crops taken after clovers, following up the ravages of Cockchafers. The first "slugworm-like" larvae of *Lema melanopa* were observed in June 16th feeding on oats. In the same field last year large numbers were noticed attacking the wheat. In 1917 "*Lema melanopa* L. was noticed as very abundant on wheat in Kent and Shropshire in July. This insect is often mentioned in connection with cereal crops abroad but in this country seldom attracts attention (1)."

Regarding the two Sawflies, *Pachynematus* and *Dolerus*—in June 1919 ten larvae were taken feeding on the leaves of wheat—they were placed on a wheat plant growing in a 9-inch pot and covered with muslin — and finally the greater number pupated. They remained undisturbed in the soil until the early summer, 1920, when from May 10th to 13th four emergences occurred. The first to emerge died but the next two were put on an oat plant under a bell-jar and soon ovipositing was in progress. The larvae from these eggs hatched, but owing to a mischance escaped and were lost. Shortly after (June 13th) a similar larva (8 mm. in length) was taken on the tall oat grass and on July 5th a fully-fed one measuring 18 mm. was found on a similar clump of tall oat. The sites where the larvae were found both in 1919 and 1920 are identical.

Among the Diptera, *Agromyza nigripes*[1] was first observed on June 17th when eight larvae were found mining the leaves of three wheat plants. In 1919 they were observed feeding exclusively on wheat plants, but in 1920 they seemed to have extended their sphere of activity and numbers were found on oats and couch grass. Larvae taken in June

[1] Very kindly identified by Mr J. E. Collin.

1919 pupated after about three days in the top $\frac{1}{4}$ inch of soil and emerged in May 1920; this would indicate that there is but one brood per annum. Field observations, however, showed some almost fully-fed larvae in June and some very small ones feeding about a month later —possibly there are two broods in a period of about eight weeks.

A single larva of *Rhyphus fenestralis* was taken with some larvae of *Pachyrrhina imperialis* feeding around the roots of italian rye grass in June. Cameron records it in decaying vegetable matter and one specimen at the root of *Senecio Jacobea*.

Of the Lepidoptera, *Odonestis potatoria* and *Arctia Caia* were taken feeding on grasses; the former is a general grass feeder, mostly taken on cocksfoot, the latter is a frequenter of waste places feeding, *inter alia*, upon *Lamium* spp. and cocksfoot. I have taken the latter in one instance feeding on seedling crucifers in frames though it is not generally observed as attacking farm crops.

Hepialis larvae are very common on waste places and amongst grass, they feed on any roots, and according to Theobald they are "found during winter in hop-stacks and devouring the roots of grasses, where they are extremely difficult to destroy." I have taken them at the roots of rye grass and clover in April. The soil was medium loam containing abundance of root fibres.

The Tortrix larva as mentioned under "Winter Period" was again observed on italian rye grass and in several instances feeding on oat leaves. It was invariably taken singly and probably does little damage.

VI. NATURAL ENEMIES.

In the course of my work I have reared two parasitic Hymenopterons, one from *Agromyza nigripes* and one from *Cecidomyia destructor*; so far, however, I have been unable to identify them. Most soil larvae are subject to the attacks of various insect parasites and Roberts notes instances of Proctotrupids emerging from larvae of *Athous haemorrhoidalis* and mentions parasites of *Agriotes obscurus* being observed probably "also referable to the family Proctotrupidae." I have kept a close watch on wireworms in captivity but have obtained no parasites.

Carnivorous ground beetles such as *Steropus madidus* and *Nebria brevicollis* are said to be enemies of wireworms and in both adult and larval stages attack many soil larvae. In one instance I added to a pot of soil eight wireworms and one larval *Steropus* spp. and subsequent examination showed but four wireworms all within the bottom 2 inches of soil. As escape was impossible it is safe to conclude the missing wire-

worms were destroyed either by the Carabid larva or by the remaining wireworms.

Examination of a lark's nest containing fledglings showed the following insects dead or badly damaged:

4 adult *Tipula oleracea.*
2 larvae of *Tipula* spp.
1 ,, *Apamea* spp.
3 "Surface larvae."

Jackdaws probably do much good in destroying insects, slugs, etc. One family consisting of, probably, two adults and three young birds visited a "seeds" field, after the crop had been cut, persistently for about a week at the end of June, evidently searching for slugs, beetles and insects in general. Hooper(8) gives an instance of a jackdaw being shot, when taking food to his brooding mate, the beak contents on examination revealing thirteen wireworms, four grubs and a few other insects.

VII. SUMMARY.

1. Insects which are parasitic alike on grasses, cereals, and other crops will, when these latter are unavailable, take up their quarters in the grasses and plants growing in the situations herein mentioned and, when the crops are again available return to them and commit their ravages.

2. Observations were conducted over a period of nine months on certain insects which attacked both grasses and cultivated crops in a limited area in Shropshire, viz. the farm of the Harper Adams Agricultural College, near Newport, and the immediate neighbourhood.

3. The observations indicate a danger of harbouring pests among the vegetation on headlands, hedgerows and waste places; grasses in particular, being susceptible to their visitation, are decidedly important factors in tiding pests over from season to season and this in itself suggests the advisability of keeping hedgerows and headlands clean.

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STUDIES ON THE APPLE CANKER FUNGUS. I. LEAF SCAR INFECTION[1]

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(With 2 Text-figures and Plate III.)

For some years an investigation of the biology of the apple tree canker fungus has been proceeding at Long Ashton (1), and the work has now reached a point when the results can be published in detail. This paper will be confined to the infection of the leaf scars by the fungus. The ultimate object of the investigation was to find some means of control of this disease, and as a preliminary step it was necessary to find out how the fungal parasite entered the living tree.

Only incidental references are made to the literature of the subject as a summary of the work already published is given by Cayley (2).

The apple canker fungus is usually spoken of as a "wound parasite," and it is commonly supposed that, provided no agency is allowed to wound the host, infection is impossible. In the case of the canker fungus this view needs modification, since this fungus enters the normal healthy tree without any previous external injury. In fact, a most important path of infection is through the scars left by the fallen leaves. *Nectria ditissima*[2] therefore is only a wound parasite, if we include in the term "wound" such natural ruptures of external tissues as those occurring in the normal growth of the plant.

INCIDENCE OF LEAF SCAR INFECTION.

The infection of canker through leaf scars is responsible for a large percentage of the canker at Long Ashton. This type of infection occurs on many varieties and is regular in its incidence, whilst from material received from South Devon, Worcester and Somerset, it would also appear to be widely distributed. In cider orchards (which under normal

[1] A grant in aid of publication has been received for this communication.

[2] No opinion is expressed regarding the name of *Nectria galligena*, Bres. recently suggested for the canker fungus.

conditions are not pruned) some of the varieties canker very badly and in these cases leaf scar infection is almost wholly responsible.

The association of canker with buds has been pointed out by Goethe (3).

Excellent material for studying this method of infection was afforded by some trees raised in the course of fruit-breeding work at Long Ashton. They were seedlings of the cross Kingston Black × Médaille d'Or, two cider varieties, and developed canker very freely. In August 1919 numerous examples of young cankers evidently started from buds formed in the preceding autumn were found and these left no doubt that the canker fungus could enter the tree by some means in the region of the buds. Continuous observations have since been made on these trees.

The first point noticed was that the cankers on the 1918 wood were considerably in excess of those formed in the previous years. Counts of the cankers on the wood formed in five consecutive years gave the following results: on the 1915 wood, 1 canker; on the 1916, 4; on the 1917, 29; and on the 1918, 434. The number of 1919 was over 600, but exact figures were not obtained. On these trees, infection of the shoots took place during the year succeeding their formation, viz. the 1915 wood was infected in 1916, the 1916 in 1917 and so on. The trees in question had never been pruned and therefore the number of cankers formed on the growth of any one year gave an indication of the number of infections occurring during the following season.

The number of infections in some cases was exceedingly high. Taking a single branch of one of the trees, not more than $1\frac{1}{2}$ ins. in diameter at its base and about four to five years old, almost every bud of the 1918 wood on that branch was cankered, the numbers reaching the very large total of 65. Besides the actual cankers, there were cases where the buds of 1918 failed to develop in the spring of 1919 and were possibly killed off by the fungus or at least so injured as to be unable to develop whilst the main stem remained uninfected. Such shoots, during August, showed long stretches of bare stem, and a tree severely attacked presented a partially defoliated appearance.

Large numbers of bud infections having been observed on the trees above mentioned, search was made to find similar infections on other varieties. Leaf scar infection was found to be extremely common, in fact so widespread as to give the impression that it would affect any variety provided the conditions were favourable. It has been found on Warner's King, Bramley's Seedling, James Grieve, King of the Pippins, Lord Suffield, Devonshire Quarrenden, White Transparent, Royal Jubilee and many other dessert and culinary varieties and also on many

cider varieties such as Kingston Black, Cap of Liberty and Strawberry Norman. There is, however, marked difference in the susceptibility of varieties. A striking instance of this was afforded in one of the plantations at Long Ashton where varieties growing adjacent to each other have behaved in characteristic manner during the last two winters. A record of the number of new infections occurring on the different varieties in the winter 1919–20 was as follows: Cox's Orange Pippin, no bud infection; King of the Pippins, 49; Worcester Pearmain, 1; Devonshire Quarrenden, 149; Beauty of Bath, nil. A particularly interesting point about this result is that Cox's Orange Pippin is usually regarded as extremely susceptible to canker, whilst Worcester Pearmain is by no means a resistant type. In view of the normal susceptibility of the King of the Pippins it is interesting to note that in another plantation a little distance away a row of this variety showed no canker at all. Anyone drawing conclusions from the behaviour of the trees on the above-mentioned plot would be completely misled as to the susceptibility of the varieties concerned. The explanation of this behaviour is at present obscure, but there are obviously many factors, such as, for instance, the vigour of the tree, the influence of the rootstock, the condition of the soil, or some inherent quality of the sap, about which we know very little at present and which may supply the key to the mystery.

The period of infection in the case of the Kingston Black × Médaille d'Or seedlings is limited almost completely to the spring. The first infection of the 1919 wood of these seedlings was found on March 31, 1920, but after this date infection proceeded rapidly. During the winter 1920–21 three infections of the 1920 wood were found as early as February 1921. On most varieties, however, a large percentage of the infection takes place in the early autumn immediately after defoliation. During the last autumn (1920) infection of the lowest leaf scars of that year's growth occurred in many cases before the upper leaves had fallen. The rapidity with which infection took place was very striking, in some cases practically every shoot being attacked before defoliation was half completed. Generally speaking, therefore, there are two periods when leaf scar infection is especially active—(*a*) in the early autumn, and (*b*) in the spring. It is not suggested, however, that infection entirely ceases at other times of the year, in fact it appears to continue throughout the summer and with certain varieties during the winter, but the spring and autumn are periods when infection is markedly prevalent.

Comparing the severity of the attack of the winter 1919–20 with that of 1920–21, it would appear that the latter season was specially

favourable to the fungus. For instance, the first examples of leaf scar infection of the 1919 growth were not found until January 3, 1920, when three cases only were discovered in a plantation of 230 trees of 22 different varieties. In 1920–21 the intensity of infection varied very much, but some of the trees were very severely attacked as early as September 23. It is probable that weather conditions influence the incidence of canker as well as other diseases and one of the conditions most favourable to its spread seems to be rain, which was excessive during the early autumn of 1920. On the other hand, during the previous summer, the trees suffered from a severe attack of aphis and this undoubtedly weakened the trees and possibly rendered them unable to resist infection.

Whilst, as already pointed out, infection may take place on partially defoliated shoots in the autumn, at the scars of the recently fallen leaves, yet no scar infection has been observed so long as the leaf remained attached to the shoot at that point. The most vigorous trees of each variety retained their leaves longer than the weaker ones and were usually more resistant to canker. Cox's Orange Pippin, Worcester Pearmain and Beauty of Bath each retained their leaves till the end of October. The Médaille d'Or × Kingston Black seedlings referred to above also held their leaves till very late. Some attempt was made to correlate the periods of defoliation with susceptibility to canker and although, generally speaking, those varieties which are very susceptible defoliate early and those which are resistant are on the whole late, the differences between the two do not appear to be sufficiently marked to warrant any significant conclusion.

An interesting point worthy of notice is that the varieties which are susceptible to canker frequently show a similar susceptibility to *Fusicladium dentriticum*. This correlation is independent of the infection of scab wounds by Nectria, an account of which will possibly be published later. One sometimes finds shoots which are scabbed down one side of the stem only and in the shoots of King of the Pippins and Devonshire Quarrenden, the scabbed sides also bear numerous leaf scar infections. The conditions governing scab infection must evidently be similar to those governing leaf scar infection of canker. The side of the stem attacked by the scab and canker fungi is usually that exposed to the prevailing winds, which at Long Ashton come from the south-west. It may be that the lowering of the temperature of the exposed side of the stem retards the formation of the protective phellogen just sufficiently long to allow the fungus to infect.

In addition to infection of the recently formed leaf scars, occasional infections of a stem two or three years old take place in the region of a dormant bud. Such infections are readily distinguishable, at least in their early stages, by their similarity in appearance to the infections on the young wood, by the lack of any swelling of the stem and the absence of concentrically arranged fissures which are so characteristic of cankers of long standing.

STAGES IN THE DEVELOPMENT OF LEAF SCAR INFECTION.

In order to find out how the infection is brought about, search was made for the early stages of bud infection.

The seat of infection appears to be associated with the base of the bud rather than the interior of the bud itself. The earliest stages of infection are sometimes not visible externally at all and the bud has a normal appearance. It is only on cutting the stem that the infected tissues can be discovered and in many cases it is found that a small lateral portion of the leaf scar is infected, although the colour of the bark and the appearance of the bud are perfectly normal.

The first visible external signs of any disorder varies in different cases, but is usually associated with a small crack in the leaf scar. The sap of the apple stem quickly oxidises to a bright reddish brown colour on exposure to air and this property of the sap enables small injuries to the leaf scar tissue to be detected fairly readily. On strongly grown shoots, such as the "leaders" springing from the crown of the trees, no visible split develops at all, the first stage of infection being seen in the formation of a circular dark reddish brown spot on the main stem at the extreme edge of the leaf scar. This spot, at first the size of a pin's head, increases rapidly to about the size of a pea.

The next definite stage in infection is the development of the primary scar, *i.e.* the scar first limited by the newly formed phellogen. This development arises rapidly and varies in position according to the exact spot where infection first took place; it is usual, however, to find it rather below the leaf scar and more often in a lateral position than a median one (Plate III, Fig. 1). When once the fungus has entered the stem, growth is extremely rapid and it is only a short time before the stem is completely encircled. The tissues of the bud being young and succulent are very quickly attacked by the fungus, so quickly indeed that it is often difficult to obtain a young leaf scar infection in which the bud itself is not infected with mycelium. The separation of the cuticularised layers of the bark from the underlying cortex frequently occurs, and these form a

ragged membrane over the scar. Pustules of conidia are sometimes found on the primary scars in January and February and these show the extraordinary vigour with which the fungus flourishes during the dormant period of the host. Plate III, Fig. 2 shows young infections on the Médaille d'Or × Kingston Black seedlings, which only become infected in the late spring and the cankers therefore develop slowly. Typical leaf scar infections as they appear in summer are shown in Plate III, Fig. 3.

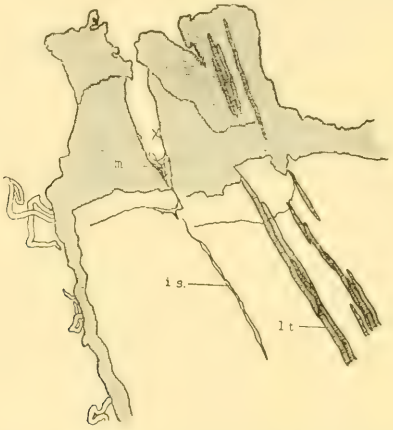
The development of the canker after bud infection may be so rapid that the whole of the shoot above it is killed almost immediately. On the other hand, development may be slow and the canker may take three or more years before it completely encircles the stem. It is possible to find many cases of cankers on old wood which can well be attributed to leaf scar infection (Plate III, Fig. 4).

MICROSCOPIC DETAILS.

Observation of the development of the canker showed that in many cases at least, the infection started from the leaf scar and not from the bud itself. The leaf scars were therefore examined minutely. The dead tissues of the leaf scar were invariably found to be infested by fungi, one interesting fungus not yet identified being almost universally present. It has been found on the leaf scars within a week of defoliation and very rapidly gains a foot-hold on the scar tissue. It has dark coloured mycelium and is associated with a pycnidium containing dark, olive green, muriform pycnospores (Text-fig. 2). The pycnidia are sometimes immersed in the leaf scar tissue or they may occur outside seated on a small mass of hyphae. In addition to the pycnidia, dark coloured conidiophores are commonly found bearing spores similar to *Fumago vagans*, but whether these fructifications belong to the same fungus as produces pycnidia is unknown. The fungus has been noted upon specimens from Cambridge and Worcestershire and is probably of common occurrence. Whether this fungus plays any part in bringing about infection is not known, but such a possibility cannot be overlooked and therefore its occurrence is recorded here.

In the infection of leaf scars in the early autumn, no case of infection was discovered whilst the leaf remained attached to the tree, although numerous instances were found but shortly after defoliation. Inoculation experiments through the scars of freshly removed leaves during June 1919 were completely unsuccessful, although two inoculations of wounds with the same inoculant produced typical cankers. At the normal fall of the leaf in the autumn, however, the host is not in as active state of

growth as in June. When a leaf falls, the tissue exposed is submitted to the desiccating effects of the atmosphere and results in the drying up of the cells exposed. The contraction of the tissue which takes place on drying results in small cracks appearing in the leaf scars, especially in the region of the soft tissue adjacent to the leaf traces. These small cracks allow the canker fungus the opportunity to enter the host, which it does very readily, before the host has had time to form a phellogen (Text-fig. 1). The small depressions between the leaf scars and the main stem hold up small quantities of water which probably aid the germination of the spores of the fungus. The tissue of the leaf base is rather looser than the normal cortex and the canker fungus grows very freely in the intercellular



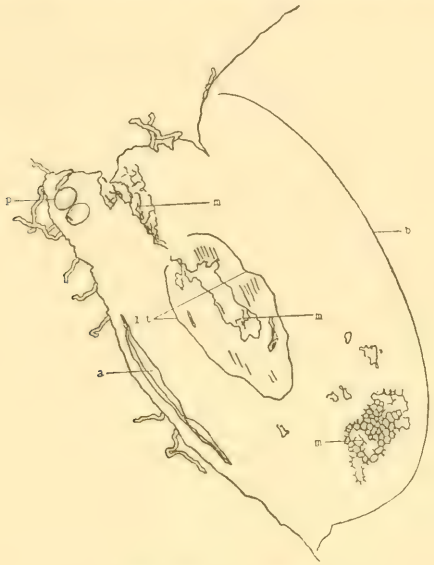
Text-fig. 1. Radial longitudinal section of a leaf base showing a crack in the leaf scar and the mycelium about to enter the host. The intercellular space in the tissue below the crack affords an unimpeded entrance to the fungus. ×34. *m* = mycelium; *l.t.* = leaf trace; *i.s.* = intercellular space.

spaces. The spread of the fungus is helped by the slow response of the host to form a limiting phellogen, and the stem soon becomes completely girdled (Text-fig. 2).

The second period of infection, which takes place when the trees become active in the spring, however, is very similar in its symptoms to the autumn infection. Many tiny cracks in the leaf scar tissue are found especially towards March and April when the buds are bursting. Throughout the winter the buds gradually swell by normal growth, but the swelling is usually sufficiently slow to allow the continuity of the phellogen to be maintained. The growth cracks, however, frequently extend to considerable depths. With the enormous increase in growth in early

spring, fissures arise which expose the tissues of the leaf base and readily allow the canker fungus to enter. The host only slowly reacts against the fungus which therefore quickly develops, in many cases surrounding the stem and killing off the shoot above it.

The view that the cracks resulting from the growth of the buds, afford a means of entrance to the fungus in the spring, is also strengthened by the observation, given above with regard to the times of appearance of bud infection. The Médaille d'Or × Kingston Black seedlings are



Text-fig. 2. Radial longitudinal section showing infection of the leaf base and the crack through which the fungus first entered. There is no sign of phellogen formation and the mycelium has penetrated deeply in the intercellular spaces. An infection of *Venturia inaequalis* can be seen on the outside of the stem. ×25. *m* = mycelium; *l.t.* = leaf trace; *b* = limit of infected region; *p* = pycnidia; *a* = *Venturia inaequalis*.

naturally exceptionally late in bursting into leaf, but until this takes place only very occasional infections can be found on the previous season's growth. It appears that there is a definite correlation between the appearance of canker and the swelling of the buds on the Kingston Black × Médaille d'Or seedlings and this fact points to the conclusion that the growth cracks which accompany bud development allow the entrance of the fungus.

A point of interest which may be mentioned here is that further infections of the canker fungus on the Kingston Black × Médaille d'Or

seedlings do not always develop into cankers, owing to the formation of a phellogen which excludes infected material in exactly the same way as has been found to exist when shallow cuts are inoculated artificially. Such excluded material can be recognised hanging on to the edge of the infected leaf scar (Plate III, Fig. 2).

CONTROL CONSIDERATIONS.

The economic loss by the leaf scar infection is considerably reduced in the case of bush trees by the practice of winter pruning. In this process, most of the infected wood is cut out, although some is occasionally overlooked, and also infection may take place after the pruning has been done. In the case of young trees the loss is especially important, as a large quantity of new growth is required to build up the main stems. A few bud infections on a young tree usually means a considerable loss to the tree. At Long Ashton not a few cases of grafts of varieties so resistant as that of Bramley's Seedling, have been totally destroyed by infection of the leaf scar. With orchard trees, where little pruning is done, this type of infection is most serious, and it is probable that in those cases where the trees suddenly canker all over, it is leaf scar infection which is responsible for the damage. The sudden increase in the number of infections on the Kingston Black × Médaille d'Or seedlings, as recorded above, shows that such a state of things does occur.

There does not appear to be any valid reason why spraying of the leaf scars, especially before the buds burst, should not prove a successful control measure in the case of spring infections of the canker fungus. Preliminary trials(4) with this end in view were started in December 1919. Twenty branches were selected from the Kingston Black × Médaille d'Or seedlings and ten of these branches were sprayed with copper stearate on December 16, 1919, the remaining ten being left as controls. Counts made on May 23, 1920, showed that the number of new cankers on the sprayed branches was reduced from 299 in 1919 to 62 in 1920, whilst the number on the controls increased from 135 in 1919 to 299 in 1920. Additional infections took place subsequently, but in December 1920 re-counts showed a marked reduction on the sprayed branches, the numbers then being 179 for the sprayed and 500 for the controls. These results are sufficiently promising to warrant the further trials which are now being carried out.

Reference must be made here to the spraying experiments carried out by Grubb(5) at East Malling during the last two years. By spraying with lime-sulphur or Bordeaux, in May and again in June, he was able

to record a considerable reduction of canker infections. The best times for spraying are obviously immediately after defoliation or before the breaking of the buds and it is somewhat difficult to see why spraying in May and June should be markedly effective. Until defoliation in the autumn no infection would occur on the 1920 wood and it is doubtful if spraying in May or June would affect this appreciably. As regards the 1919 wood, however, the main autumn and spring infections would also have occurred before the sprayings and would not be affected by them. But as already mentioned the leaf scar infection of the previous season's wood continues slowly throughout the summer and possibly against this infection the spraying was effective.

The existence of autumn infection of leaf scars naturally increases very considerably the difficulty of the control of the disease by spraying in the case of varieties so affected. First of all the spraying of the leaf scars immediately after defoliation is dangerous, because at that time the fruit still remains hanging on the tree in many instances and spray fluids may spot the fruit. Further, the spraying of ripe fruit with a copper fungicide is open to obvious objection, and as regards sulphur sprays as an alternative, the fact that the canker fungus does not appear to be very sensitive to sulphur rather discounts their effectiveness(6). Another difficulty in autumn spraying for canker is the rapidity of infection, which results in many leaf scars being infected before defoliation is complete. At least two sprayings at the time of defoliation therefore would appear to be necessary to control the disease even if suitable spray fluids can be found.

Although spraying treatment for autumn infection therefore is not altogether promising, other treatments for reducing the amount of infection may yet be possible. Vigorous trees usually hold their leaves longer than weakly ones and any treatment therefore which would tend to increase the vigour of the trees, may by delaying autumn defoliation reduce the risk of infection.

SUMMARY.

The infection of the apple stem by the canker fungus through the leaf scars is described.

The fungus appears to enter through small cracks which appear in the leaf scar tissues, in the autumn immediately after defoliation and in the spring when the buds are swelling.

The possibility of preventing this infection by disinfecting the leaf scars by fungicides is discussed and results of preliminary trials recorded.

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EXPLANATION OF PLATE III.

Fig. 1. Early stage in the infection of a shoot of Devonshire Quarrenden. The fungus entered at the edge of the leaf scar and the boundary of the infected tissue can be distinguished. March 1920. × 1.

Fig. 2. Infections on Kingston Black × Médaille d'Or seedlings. The infections are about three months old. At *A*, a portion of infected tissue can be seen isolated and excluded from the stem. August 1920. × $\frac{2}{3}$.

Fig. 3. Typical leaf scar infections on Tom Putt, Middle Winter Pippin and an unknown variety of apple. Note that each scar surrounds a bud. August 1920. × $\frac{3}{5}$.

Fig. 4. Cankers on the 1917 and 1918 wood of Bramley's Seedling, probably developed from leaf scar infections. August 1920. × $\frac{1}{2}$.

(*Received May 2nd*, 1921.)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

ON THE LIFE HISTORY OF "WIREWORMS" OF THE GENUS *AGRIOTES*, ESCH., WITH SOME NOTES ON THAT OF *ATHOUS HAEMORRHOIDALIS*, F.[1]

PART II.

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(With 4 Text-figures and Plate IV.)

EXTERNAL STRUCTURE OF *AGRIOTES OBSCURUS*, L.

The first part of this paper contained an account of the biology and life-history of *Agriotes*. This part contains descriptions of *Agriotes obscurus*, L. in the oval, early and late larval stages, together with observations on the pupa. The stages are taken in their natural order, but since an acquaintance with the later stages for the sake of brevity has been presumed in describing the early ones, it may perhaps be necessary to refer to the general description of the final instar in some cases. In addition, it has been considered desirable to describe the mouth parts and spiracles of the larva in greater detail than was possible in a general description. Separate sections for each of these have therefore been added.

In the third part of the paper it is hoped to give some description of the larva of *Agriotes sputator*, L. with some notes on the early stages of *A. sobrinus*, Kies. = *acuminatus*, Steph. and *Athous haemorrhoidalis*, F.

I must again express my gratitude to Dr Russell and the Committee of the Lawes Agricultural Trust for the facilities given me at Rothamsted for carrying on the earlier stages of this part of the investigation, as well as for the figures reproduced in the plate. The later stages have been completed at Cambridge through the kindness of Prof. Stanley Gardiner, to whom my thanks are also due. I wish further to express my indebtedness to Mr C. Forster Cooper and Dr Hugh Scott for kindly allowing me during the last six months to work in the Cambridge Zoological Museum and to make use of the books and collections there.

[1] A grant in aid of publication has been received for this communication.

The Egg.

Generally broadly ovoid, but irregular in both shape and size. Average dimensions of four ova ·59 mm. × ·47 mm. A fifth measured after fixation ·64 × ·40. Shell transparent, exhibiting the milky-white yolk and embryo within. The germinal band of the embryo appears as a yellowish-green stripe. The surface of the shell is almost smooth, though a few irregular shallow punctures are visible under the microscope.

First Larval Instar.

The young larva on hatching is about 2·5 mm. in length and less than ·4 mm. in breadth across the prothorax. It is milky white in colour, shining, but when examined under the microscope is found to be minutely punctured and wrinkled. It has the general appearance of being broader and more stumpy than the later stages. Only the mouth parts are yellow, the mandibles being darker still and quite brown at their apices. The 9th abdominal segment is pointed and somewhat constricted at about two-thirds of its length. All the setae are colourless, the eye spots black. The head and all the body segments, with the exception of the 9th and 10th abdominal segments are broadest in the middle and somewhat rounded at the sides. The cauda and margins of the sensory pits of the 9th abdominal segment are colourless, so that the latter are difficult to make out in life. The marginal striae at the base of the body segments, though present, are also only to be made out with difficulty.

Throughout the first instar the larva remains pale and semitransparent, though at the end its general colour has become faintly yellow. The margins of the sensory pits on the 9th abdominal segment are then visibly brown, the constriction near the end of the same segment has been lost and the anterior half of the segment has its sides subparallel, while the posterior tapers to a point at the cauda, which is still colourless or almost so. Five larvae taken from the pots at this age measured 3·25–3·5 mm. in length.

The following points may also be noted in which the young larva of the first instar differs in greater or less degree from the full-fed larva.

Sides of the head subparallel. It is broader than long, measuring the breadth across the middle and the length from the insertion of the mandibles to the base of the head.

Instead of the three-pronged nasale or clypeal process, the latter is represented by a single-toothed process, twice as broad as long and blunt at the apex (Fig. 2 *e*).

In the antenna the 3rd or supplementary segment is proportionally longer than the same segment in full-fed larvae, being two-thirds the length of the 1st and 2nd segments combined.

The mandibles are broader in proportion to their length than those of the full-fed larva, the width at the base being rather more than two-thirds of their length. The apex is finely pointed and considerably incurved. The eye spot is pitchy, situated somewhat further from the base of the antenna than in the full-fed larva and is more conspicuous.

The nervous ganglia, which are plainly visible through the integument in stained preparations, are in proportion to its size very large in the young larva. The two lobes of the supra-oesophageal ganglion, situated in the prothorax and extending backwards into the mesothorax are especially noticeable. There are thirteen ganglia in all, as is common in coleopterous larvae. The spiracle itself is rather more rounded in the young larva, the breadth across the two orifices being greater in proportion to the length than in older larvae. The teeth on either side of the stigmatic orifices are few in number, being about seven or eight in the thoracic and six in the abdominal spiracles.

At first the peritreme is almost colourless, but later it becomes yellow, though it is less strongly chitinised than in older larvae. Each orifice is bordered separately by its own peritreme, so that the two orifices have the appearance of separate spiracles with a small interval between them. At the anterior end of each the peritreme disappears and the boundary to the orifice is merely the unthickened cuticle.

The hairs situated on the tergites between the mesothoracic and 8th abdominal segments are pale, the posterior row slightly longer than the length of the segments to which they belong, the anterior short. In the prothorax, however, where the segment itself is longer than the other segments, the hairs in both rows are considerably shorter than the segments and are about equal to one another. On the 9th segment the hairs surrounding the apex are noticeably long and the posterior hairs of the head are also long, being longer than the anterior hairs and as long as those on any other part of the body.

Second Instar.

As already stated (Pt. I, p. 126) the first ecdysis takes place in June. The larva is then of about 3·5 mm. in length. In the second instar its growth is much more rapid and at the end it has attained a length of 5·5–6·5 mm. and breadth of about ·5 mm. The colour of the larva is now pale yellow and the body is quite opaque. The tergites are nearly smooth,

but under the microscope can be seen to bear numerous short irregularly shaped striations. The sternites are smoother.

The segments of the body are cylindrical or nearly so, but the head is flattened dorso-ventrally and is somewhat darker in colour than the remaining segments.

The setae are slightly yellowish and all are shorter than the segments to which they belong. The setae of the posterior row are longer than those of the anterior, as in older larvae.

The marginal striae at the base of the 2nd and 3rd thoracic and of the abdominal segments are very fine, but are visible under the microscope. Similarly, those forming the anterior and posterior margins of the prothorax.

The nasale, or clypeal process, is now trifid, with a stout median dens and a smaller lateral dens on either side of the median one, scarcely half as long and little more than half as broad as the median one. Ventral to the nasale the semi-lunar process over the mouth, described in the older larva, can now be identified. In the specimen in which it was found four or more teeth could be seen, the remainder being represented by the sinuate margin of the process. The proportion of length to breadth of the mandible now nearly corresponds to that of the full-fed larva. In the antenna the dorsal process at the apex is still proportionately longer than in the late stages, being about one and a half times the length of the 2nd segment.

The spiracles are now distinctly margined by a brown peritreme, which however is pale and very narrow on the anterior margin of each respiratory orifice. The posterior margin is also narrow but more distinct. The lateral margin and also the central septum are broader than those of the first instar and furnished with pittings or corrugations corresponding to the number of teeth which project from the sides of each orifice. These number eight or nine in the abdominal and eleven in the thoracic spiracles.

The stigmatic scar is visible as a colourless strand in the chitin placed at an obtuse angle to the axis of the spiracle and dorsal to it. The inner end is attached to the atrium, while the outer terminates in a slightly divaricate fork in the cuticle.

The 9th abdominal segment is subparallel at the sides for nearly two-thirds of its length. It then tapers sharply to the apex, from which the cauda is produced. The latter is distinct, sharply pointed and in colour tinged with yellow.

The sensory pits are bordered by an ochraceous rim which is elevated

above the surrounding area chiefly at the sides. Behind, it is less raised and paler in colour.

Third Instar.

The second ecdysis occurs at the end of July or in August and the larva passes its second winter in the third instar. At the beginning it is about 6–8 mm. in length and $\frac{1}{2}$ mm. in breadth. In colour it is distinctly yellow and in other respects also except size closely resembles the full-fed larva.

The tergites are sparsely punctate but bear numerous irregular striations, which may be seen under a low magnification.

The sternites are smoother. Setae distinctly yellow. Nasale trifid, with median dens the stoutest. Dorsal process of antenna about equal to the second segment.

Spiracles are now distinctly different from those of *A. sputator* at the same age, being shorter and proportionally wider.

The teeth or corrugations at the sides of the orifices number eight to ten in the abdominal and fourteen in the thoracic segments.

The stigmatic scar is thicker than in the previous instar.

The 9th abdominal segment is broadest just posterior to the sensory pits and from that point gradually tapers to the distinctly pointed cauda. Sensory pits margined on all sides with brown.

The following table gives some idea of the actual increase in length of the larva during the early instars. All the larvae hatched in 1916 (or 1918) and were afterwards kept in pots under normal conditions as far as possible.

Instar	Date of observation	Average length mm.	No. of specimens	Range of length mm.
First (1st day)	Aug. 1916 (1918)	2·5	5	2·0 — 3·0
„ (9 months)	May 1917	3·35	5	3·25— 3·5
Second	End July 1917	5·75	6	4·5 — 6·5
Third	Mid Sept. 1917	6·82	7	5·75— 8·5
Fourth	End July 1918	9·25	2	8·5 —10·0
Fifth	End Aug. 1918	10·46	14	8·25—13·0
. . . .	. . . . .	. . . .	. . .	. . . .
Eighth	Early July 1920	17·0	3	16·0—18·5

If this table of records is compared with the estimate made in Part I of this paper (p. 127) it will be seen that the larvae were about a year behind the estimate in 1920. It seems likely, therefore, that the length of life in the larval stage is, or at least may be, of six years' duration[1].

[1] Some confirmation of this forecast has been obtained since the above was written. Of three larvae of the 1916 brood, on August 22nd, 1921, one was found to have developed into an imago (♀), a second remained a larva (of length 22 mm.), the third was not found, possibly having attained maturity and so having been overlooked in the soil.

General Description of the larva in Final Instar.

Length 22–26 mm.; breadth 1·75–2·0 mm.

Apart from the head and 9th abdominal segment nearly cylindrical: yellow, usually rather pale.

Head quadrangular, slightly broader than long, flattened above and beneath; somewhat darker in colour than the remaining segments. Wider behind than in front, bearing a long seta near each of the four angles, besides several shorter ones situated chiefly anteriorly; punctures sparse and irregular.

Upper surface with an elongate-oval plate (the cephalic plate), which is expanded anteriorly over the base of each mandible. Anterior margin of the plate, over the entrance to the mouth, with a strongly chitinised brown process, which divides into three sharply pronged denticles, the middle one being the longest. Anterior angles of the head slightly rounded.

Antennae situated close to the base of the mandibles, short, consisting of two segments and two processes borne separately at the apex of the 2nd segment. Ventral process shorter, conical and nearly colourless; dorsal process darker and more strongly chitinised, linear and about equal in length to the preceding segment. Probably the latter should be regarded as the 3rd segment.

Eye spot dark brown or black, situated laterally, a little below the base of the antenna.

Mandibles rather small, stout, yellowish brown, with sharply pointed, pitchy black apices; each bearing two denticles on the inner margin and a brush-like process, the penicillus, at the base.

Ventral surface of the head with a pair of somewhat oblique furrows, brown, nearly straight and reaching from the base of the mandibles to points beyond the base of the hypostome, also with two ridges of darker brown chitin, somewhat bowed, situated in the cavity between the epicranial plates (genae) and the maxillae and nearly reaching the posterior apices of the cardines.

Maxillae (Fig. 1) each with a very short cardo, articulating with the tentorium at its second branch. Stipites somewhat rounded on their outer margins and straight within. Palps yellowish-brown, composed of four segments and borne on white membranous palpigers at the apices of the maxillae. Galeae composed of two segments, with a small thimble-shaped process at the apex. Laciniae small, triangular, sharply pointed and densely margined with wavy hairs on their inner margins. Terminal

lobe of the *labium* approximately pentagonal, wider in front than behind, bearing two long yellow setae. Labial palps two-jointed, the first segment with several setae near its apex. Between the bases of the palps, the margin of the labium is usually somewhat produced and probably represents the ligula. Mentum transverse, broader than the submentum, almost membranous. Submentum almost tongue-shaped, usually strongly

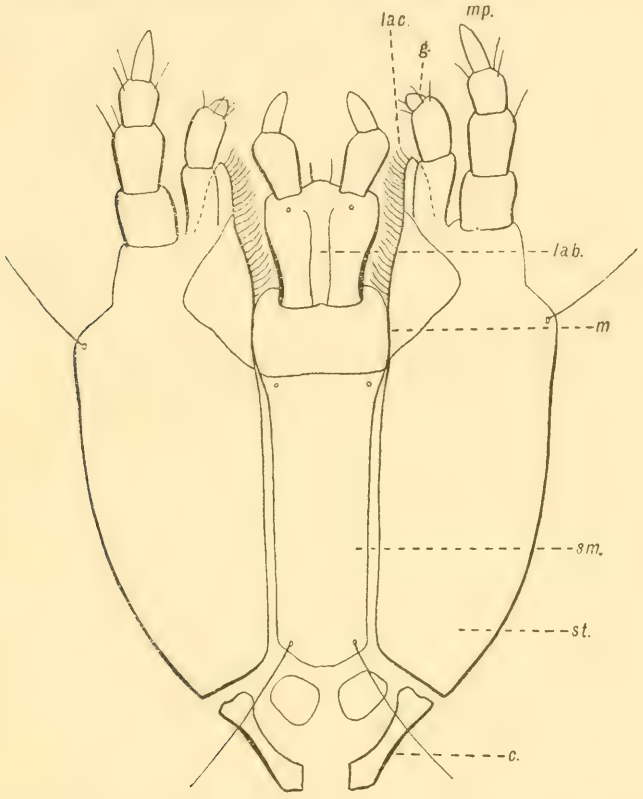


Fig. 1. Hypostome of larva (*R. Green del.*). *c* = cardo; *st.* = stipes; *sm.* = submentum; *m* = mentum; *lab.* = apical portion of labium; *mp.* = maxillary palp; *g.* = galea; *lac.* = lacinia.

chitinised, with the anterior and lateral margins nearly straight, terminating in a blunt point posteriorly. A long seta is borne at each of the four lateral angles.

Prothorax nearly as long as the meso- and meta-thorax taken together. Both anterior and posterior margins of the pronotum have a border of fine longitudinal striations, the anterior extending somewhat further than

the posterior margin. The edge of the border is defined by a transverse brown line and a transverse series of minute pores, which are almost equidistant from each other. The meso- and meta-notum, as also abdominal segments 1–8, have the border only on the posterior margin.

The pronotum, like the tergite of each succeeding segment up to the 8th abdominal segment, is separated from the pleura by a narrow suture, running from one marginal border to the next. The pleura of the prothorax is, however, nearly triangular in shape instead of being parallel-sided as in the other segments.

Prothoracic *sternum* large, pentagonal, with its apex extending almost to the anterior coxae. Sterna of the meso- and meta-thorax narrow, nearly parallel-sided but somewhat broader in the middle. All *coxae* are surrounded by striations, concentrically arranged around their bases, except on the inside. They are rather long and pale and bear on their anterior and posterior faces a number of short, stiff, brown bristles. Each is approximate to its pair. A small brown spot, having the appearance of a pore, is placed near the lateral margin of the coxa.

Each succeeding segment of the leg is considerably shorter than the coxa and the broadest little more than half its breadth.

Trochanter much longer on the inner than the outer side, *femur* on the outer than the inner.

Tibia about equal in length to the femur but more slender. *Tarsus* consisting of a single segment, narrowed at a short distance from the base into a long, sharp, almost sickle-shaped claw.

The inner margins of the trochanter, femur and tibia bear short stiff bristles like those of the coxa, evidently of use in progressing through the soil. There are also two long fine setae attached to the inner margin of the trochanter, and one to the femur, as well as a whorl of rather shorter fine hairs round the base of the tarsus. The outer margin of the leg bears only a few fine, short hairs.

On the upper surface the *cuticle* is shallowly sculptured with fine punctures and irregular sutures, its general appearance being much smoother than in that of *A. sputator* (Plate IV, figs. *b* and *c*). The area anterior to the spiracles is smooth, or almost so. There is a fine mediodorsal suture running from the anterior margin of the prothorax to the posterior margin of the 8th abdominal segment interrupted in its course only by the marginal striations already mentioned.

The tergite of each segment from prothorax to 8th abdominal segment bears two transverse rows of six rather long yellow hairs near the anterior and posterior margins respectively, the posterior hairs being

about twice the length of the anterior. The prothorax has a further row of short hairs rather variable in number close to the anterior marginal border. The ventral surface of the thorax is bare save for three pairs of short hairs on the prosternum. On the abdominal sternites 1–8 three rows are present bearing four, two, four hairs respectively.

The single pair of thoracic *spiracles* is situated in the pleurae of the mesothorax; those of the first eight abdominal segments near the lateral margin of the tergites a little behind the anterior margin (Plate IV, fig. *a*).

The abdominal segments gradually increase in size from the 1st, which is the smallest, to the 9th.

The *muscular impressions*, present on the abdominal segments of most Elaterid larvae, can hardly be made out in this genus; the longitudinal branch can, however, sometimes be seen behind and somewhat dorsal to the spiracle, running nearly parallel to the lateral suture.

The *9th abdominal segment* is considerably longer than the preceding one, conically paraboloid (as Beling (2) aptly describes it), with a pair of large open pits, margined with brown, situated one on either side near the anterior margin of the tergite (Plate IV, fig. *a*). In life they are nearly round, but contract at the sides to an elongate oval shape when preserved in spirit. Their margin of stout brown chitin is somewhat raised above the general area of the tergite on either side. Within, the pits are lined with pale membrane, which bears numberless minute dark hairs arranged over the entire surface. Similar fine hairs are also found on the inner surface of the chitinous rim just mentioned. In consequence of the presence of these hairs situated within the pits, the latter are presumed to have a sensory function, though what it may be has not yet been ascertained. Formerly, they were wrongly supposed to be spiracles, but more recently they have been referred to as muscular impressions by Henriksen (7) and Schiödte (12).

From the posterior margin of each of these pits an almost straight suture runs backward, terminating beyond the middle of the segment, slightly more dorsal than the sensory pit. Situated between these two sutures is another pair of parallel sutures, arising and terminating at corresponding points of the tergite (seen in Plate IV, fig. *b*).

The apex of the segment is brown and is slightly produced into a stumpy cauda.

The 9th tergite (Plate IV, fig. *a*) is continued uninterruptedly on the ventral surface for about half its length posteriorly, while the 9th sternite, which is an arch-shaped area and contains the pseudopodium near its apex, is separated from the tergite by a deep and brownish

suture. Within the suture is a belt of strong yellow chitin, marked transversely with striae similar to those forming the border to the other segments. The remainder of the sternite is a semi-ovoid area strongly chitinised and almost smooth. This encloses near its apex, posteriorly, the cylindrical pseudopodium, which is by some considered to represent the 10th segment. Surrounding the pseudopodium the 9th sternite is convex and margined with a concentric border of fine striae in a somewhat darker yellow area. The *pseudopodium* itself is white, fleshy and tubular and bears around its base a whorl of eight short, tapering, incurving hairs. It is situated somewhat anterior to the middle of the 9th abdominal segment. The anal aperture is linear and extends across the pseudopodium in the longitudinal axis.

The 9th tergite both above and beneath bears numerous rather long yellow-brown hairs, which are more numerous towards the apex. The 9th sternite, anterior to the pseudopodium, has a transverse row of six hairs, the two median ones of which are shorter and slightly anterior to the rest. A single short hair is also usually present on either side of the pseudopodium.

Head and Mouth-parts of Larva.

On the dorsal surface of the head there is a *cephalic plate*, expanded anteriorly over the base of each mandible, tapering posteriorly and ending in a point in the occipital region (Fig. 2 *a*). Doubtless it is composed of several fused sclerites, probably the frons, clypeus and labrum. Laterally it is separated from the epicranial plates by a deep suture. In *Agriotes*, and apparently also in *Cardiophorus*, the *epicranial plates* meet for a short distance at the base of the head (vertex) behind the cephalic plate, though in most Elaterid larvae the apex of the cephalic plate extends backwards to the posterior margin of the head and the epicranial plates are dorsally entirely separated by it. On the ventral surface the cephalic plates are fused together posterior to the base of the hypostome and though there is little sign of a suture in the median line, there is a distinctly darker line ventral to the tentorium which probably represents the line of junction. There appears to be no true gula.

In its general plan the structure of the *tentorium* is simple. On the floor of the head at the base two arms extend for a short distance on either side, almost parallel to the thickened margin of the occiput. From the occiput the main longitudinal trunk is carried forward by two beams, closely approximate, on the floor of the head to the base of the maxillae. At this point two small plates of thickened brown chitin are developed

on the tentorium and with these plates the cardines of the maxillae articulate at their posterior apices. From this point one pair of arms extend, diverging somewhat, beneath the margins of the hypopharynx to the inner margin of the mandibles. A second pair, more dorsal, extend forwards, diverging outwardly towards the large flexor tendons of the mandibles, but terminating abruptly with a number of short ligaments before reaching them.

The *antennae* are short and are situated at the anterior angles of the head, close to the base of the mandibles. They consist of (1) a stout basal segment, nearly twice as long as broad, rounded on the outer but almost straight on the inner side, and bearing a rather long tapering seta not far from the apex on the outer margin; (2) a second segment about two-thirds the length of the first and more slender, widest near the apex and bearing a short seta on the inner side of the apex; (3) two processes at the apex of the second segment, borne separately, the ventral and shorter one conical, very thinly chitinised and nearly colourless, the dorsal and longer one more slender, yellow and strongly chitinised, about equal in length to the second segment, though not more than half as broad. The apex of the dorsal process bears a long stiff seta and also three minute ones, all projecting forward, the first-named being longer than the process itself. In Carabid and Staphylinid larvae, where somewhat similar processes are found on the antennae, Kemner(8) regards the longer process as the terminal segment and calls the shorter ventral one "supplementärglied." Gahan(6) has suggested that the latter is a sensory organ.

Three pores, which appear to have a sensory function, are present in the basal segment. In the second segment a pore bearing a short spine is present on the outer margin near the distal end, while another similar pore may be found near the middle of the anterior margin.

The *eye* is small, dark brown or black, situated laterally, a little below the base of the antenna. The chitin of the head is continued uninterruptedly over it and is not raised.

The *mandibles* are curved inwards and bear on their inner surface two denticles. The first of these, situated near the apex, is flattened dorsi-ventrally and overlaps the mandible of the opposite side when the two are brought together, that of the right mandible usually, but not invariably, overlapping the left. The presence of this denticle is characteristic of the genus *Agriotes* amongst Elaterid larvae, though it is present in certain other groups of coleopterous larvae. It is somewhat rounded in outline, though its anterior margin forms almost a right

angle with the inner edge of the mandible at the point of junction, and projects in the horizontal plane.

The second denticle, also on the inner face of the mandible, is situated nearer to the base. It is much sharper than the first and is on a plane nearer to the ventral surface of the mandible, arising from a point a little within the actual margin. This denticle is called by Schiödte the *retinaculum* and is common to all the Elaterid larvae known with the exception of the section *Agripnini* (of which *Brachylacon murinus*, L. is our only British species) and the genus *Cardiophorus*.

At the base of the inner edge of the mandible there arises a small process of brush-like structure called by Schiödte and Henriksen the *penicillus*. Ford, who gives a figure of the mandible(5), has called this process the "lacinia mobilis," but it is certainly not homologous with the lacinia mobilis of some of the Malacostraca, to which the term was first applied. In them, as Mr L. Borradaile has kindly pointed out to me, the process is independently moveable, whereas the process in *Agriotes* is fixed. Its function seems to be to cooperate with the rest of the dense mass of hairs at the entrance to the mouth in preventing the entrance of unwholesome matter to the mouth. Schiödte, however, refers to it as assisting in the absorption of blood. Several hair follicles are visible on an examination of the dorsal surface of the mandible, of which one is situated about in a line with the retinaculum and almost on the outer margin of the mandible: two others are situated nearly in the median line, more basally. The first of these bears a seta of considerable length, which extends obliquely forwards and doubtless has a tactile function.

The mandible itself is of considerable thickness and somewhat excavate on its outer side, but is narrowed on the inner side to form the cutting margin. In transverse section it is therefore almost triangular. Much variation appears to exist in the mandibles of this species, but some of the apparent variation is caused by erosion, mandibles which should normally be sharp-pointed and somewhat long presenting an appearance of stumpy bluntness.

The principal condyle is ventral and articulates with the thickened anterior margin of the epicranial plate, ventral to the base of the antenna.

Between the mandibles, at the anterior margin of the compound structure which has been called the cephalic plate, there is a thickening of the chitin into a tridental process, of which the middle denticle is the longest (Fig. 2 *b*). This process is situated immediately above the entrance to the mouth and was referred to by the older writers as the clypeus, but

as it probably only represents a portion of the clypeus it is perhaps better to adopt Henriksen's term of *nasale*. This also, like the mandibles, is much subject to erosion and though the three denticles are visible as a sharply pronged trident in some specimens, in others they are worn down to a level with the base. Variation appears to take place to some extent in regard to this organ, one specimen having been found bearing four denticles.

Ventral to the "nasale," nearly apposed to it, and also extending outwardly, is another chitinous process, semi-lunar in shape and bearing

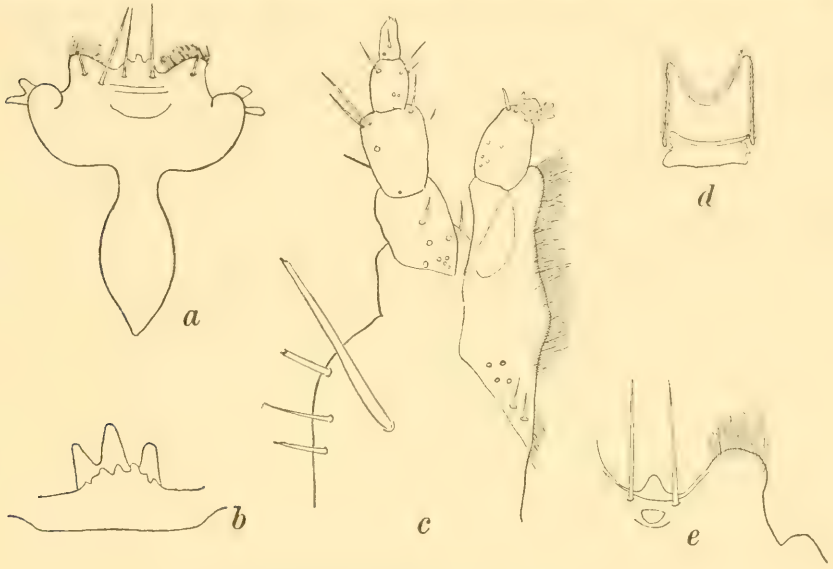


Fig. 2 a. Cephalic plate of larva.
b. Nasale and subnasal process from beneath.
c. Anterior portion of maxilla.
d. Hypopharynx.
e. Nasale of larva in first instar.

some seven teeth (Fig. 2 *b*). It does not extend so far as the nasale, the points of the teeth reaching little beyond the base of the nasale. The teeth are asymmetrically disposed and of unequal length. They may also, with the nasale, be supposed to provide a gripping surface in apposition to the mandibles. No suture has been found between the nasale and this process to indicate that it is a separate sclerite, though so much fusion appears to have taken place between the sclerites on the dorsal surface of the head, that it may possibly represent the labrum of other insects. A similar process has been found in the larva of *Dichiro-*

trichus placidus, Gyll., a Carabid, by Kemner(8). From the base of this process the chitin is continued in a fairly thick band on the palate of the mouth.

On either side of the nasale the anterior margin of the cephalic plate bears a tuft of fine yellow hairs, screening the opening of the mouth. The aperture itself is small and of a contracted oval shape. Within the mouth, on its lower surface, the *hypopharynx* is situated (Text-fig. 2 *d*). It is composed of a basal bar of stronger chitin, followed by an anterior portion, less strongly chitinised. This latter is yellow and almost quadrate in form, save for the anterior margin, which is deeply excavated in a semicircular form, leaving a slightly projecting horn at the entrance to the mouth on either side. Its surface, both dorsal and ventral, is covered with minute hairs in transverse rows. The main portion of the hypopharynx is strengthened by a rim of thicker chitin supporting its lateral margins. Its anterior margin and sides, but especially the apices of the projecting horns, bear a mass of fine yellow hairs, which doubtless perform the same function as do those constituting the penicillus of the mandible and the tufts on either side of the nasale. The apices of the horns fit up to the basal portion of the laciniae on either side, the base of the hypopharynx being slightly anterior to the base of the mandibles, but considerably posterior to the nasale. The length of the hypopharynx in a full-grown larva, measured from the exuvia, was ·28 mm., the width ·26 mm.[1]

Ventral to the hypopharynx but attached to it at the base is a semi-membranous plate, the floor of the mouth. In length it is about double that of the basal plate of the hypopharynx and therefore is considerably less than the hypopharynx itself. In breadth it extends considerably beyond the margins of the hypopharynx and near its antero-lateral margin bears a small tuft of bristles on either side in juxtaposition to those of the anterior margin of the cephalic plate just referred to. The anterior margin is somewhat bowed inwards and is bordered by minute hairs. Ventrally and almost on a level with the side margins of the hypopharynx on either side is a brownish semicircular mark, with five or six alveoli, which may be sensoria, situated in two rows on its anterior border.

[1] Miss A. M. Evans in a recent paper on the hypopharynx and maxillulae (*Journ. Linn. Soc.* 34 (1921), 429–456) figures the hypopharynx of the larva of *Campylus linearis* as representative of Elaterid larvae. She considers the anterior portions which I have called "horns of the hypopharynx" to be vestigial maxillulae. In the absence of more evidence than is at present available, I have some doubt in accepting this homology and therefore retain the term used above.

On the ventral surface of the head, bounded on either side by the epicranial plates, the oval *hypostome* is found (Text-fig. 1). It projects forward much beyond the opening of the mouth and is composed of the sclerites of the first and second maxillae. Around its margin, forming the boundary of the maxillary stipites, there is a rim of stouter chitin, corresponding to a similar but even stouter rim at the margin of the epicranial plates. The latter originates close to the point of articulation of the maxillary cardo with the tentorium and reaches almost to the base of the mandibles on either side, gradually becoming more slender as it extends forwards.

Between the two rims of thickened chitin there is a considerable flap of membrane which is ordinarily tucked in between them. At the base of the hypostome, posterior to the submentum and maxillary stipites, there is a considerable field which, save for the two pairs of small sclerites which constitute the cardines, is also membranous. The hypostome is thus capable of considerable extension either in the dorsi-ventral plane or simply forwards, the membranous margin causing it to function as a kind of pouch.

On either side of the hypostome, within the margins of the epicranial plates, there is a long oblique suture of a brownish colour, extending backwards from the base of the mandibles.

The two pairs of small sclerites mentioned above are situated near the base of the maxillary stipites. Each one of the outer pair, which is nearly apposed to the stipes, is of an irregular elongate form with its apex pointing obliquely backwards. Posteriorly it is attached to the tentorium at the second branch of the latter. The innermost pair of sclerites, each of which is nearly circular, is situated near the median line and sometimes overlaps the outer pair, the latter being somewhat beneath the general surface of the cuticle. Henriksen (p. 280) considers these small sclerites to have no importance and says that the outer one is not attached to the tentorium. As I have found it to be so attached, it must be considered to represent the maxillary *cardo*, as Ford has described it. No attempt can be made here to homologise the round sclerite. The outer margin of the *stipes* is rounded, the inner (bordering on the submentum) straight. It is yellow in colour, strongly chitinised and bears at its exterior angle, near the base of the palpiger, two longer and two shorter setae (Fig. 2 *c*).

The *maxillary palps* are composed of four segments, which gradually contract in width from the basal to the apical segment. They are brownish-yellow in colour and are borne on a whitish palpiger which generally

protrudes considerably from the anterior margin of the stipes. The second segment is longer than any of the others, though not so broad as the first. Two short setae are borne on the first segment, a whorl of four around the apex and two at the outer margin of the second, a similar whorl of four near the apex of the third, and two very short ones near the middle of the fourth. At the apex a series of ten or more exceedingly minute short bristles are present, which must make the palps extremely efficient tactile organs. In addition to the setae a number of pores are present on the palps, of about the same size as the follicles of the setae. These have presumably a sensory function and occur as follows:

Segment 1; a single pore at the base, in the median line of the ventral surface, also a cluster of six pores of varying size near the inner margin: segment 2; one large pore somewhat beyond the middle of the segment and one very small one at its base, both on the ventral surface: segment 3; two pores placed side by side on the ventral surface near the middle of the segment: segment 4; one pore on the outer margin of the segment near its base.

The *galeae* are two-jointed, the first segment being slightly longer than the second. Its outer margin is almost straight, while the inner margin is bowed outwards, almost elbowed. It is without setae and sensory pits. The second segment is rounded at the margin on either side, but the outer is longer than the inner side and the galea inclined inwards by reason of the manner of the articulation of the first with the second segment. A large sensorium is borne near the middle of the segment on the ventral surface, while four or five smaller ones are borne on the outer margin between the middle and the base. The apex of the segment is surrounded by a whorl of six short thick setae and within the whorl a colourless thimble-shaped process is enclosed. This process is inclined inwards and is barely one quarter the length of the preceding segment. It is covered with rounded, colourless scales.

The *lacinia* lies somewhat dorsal to the galea and is partially overlapped by it. It is flattened, composed of a single segment with the apex bluntly pointed, almost triangular and reaching a little beyond the first segment of the galea. On its outer margin it articulates behind (*i.e.* dorsally to) the base of the galea, while its inner margin is continued posteriorly to a level with the opening of the mouth, at the base of the terminal lobe of the labium. The whole inner margin and apex is almost concealed by a dense mass of wavy yellow hairs, which arise for the most part near the outer margin of the organ and protrude into the space before the mouth. At the base, near the angle of the inner margin two

short setae are situated and above them eight or nine pores of varying size.

The *submentum*, which constitutes the basal piece of the labium, is strongly chitinised, elongate with parallel sides and the base bluntly pointed. It extends from a point level with the bases of the maxillary stipites to the point of insertion of the lacinia. Its anterior margin is almost straight and it bears four long tapered setae in pairs at its anterior and posterior angles. Many small pores are found on the surface.

The *mentum* is membranous, nearly colourless, transverse and joined at its sides for about half the length to the inner margins of the laciniae. The anterior half is free. It is joined to the submentum just posterior to the level of the insertion of the laciniae and is produced forwards nearly to the level of the insertion of the galeae. The mentum is broader than the base of the *terminal lobe* of the *labium* which succeeds it but the angles are rounded off.

The terminal lobe itself, composed of the fused palpigers and ligula, is nearly pentagonal in shape. The labial palps are situated one on either side of the anterior margin, while between them the margin protrudes forwards a little in a pale rounded projection, which is evidently the ligula. Further back the palpigers may generally be roughly traced by the darker colour of the cuticle on either side, that overlying the ligula being pale and extending almost to the base of the apical lobe.

A pair of setae are situated dorsally near the apex of the ligula, projecting forwards and a second pair occur ventrally a little posterior to the first. The longest pair are placed one on each palpiger at some distance from the base of the palp, while a fourth pair of quite short spines are found not far from the base of each palpiger.

In addition to the setae there are a number of pores on the ventral surface of palpigers and ligula, as follows: a row of three between the two long setae of the palpigers, a single unpaired one on the left side just behind them, and a pair behind the setae, followed by a single unpaired median pore, while yet another pair occur almost in a line with the short basal spines, one on the outer side of each. All these pores appear to have a rather wide rounded margin with only a very minute aperture (or perhaps a sensory pin) in the middle.

The *labial palps* are each composed of two segments. The basal segment is stout and considerably rounded at the sides, especially on the inner side. It is yellowish like the general surface of the cuticle, but is paler at the apex. The apical segment is only about half the breadth of the basal segment, brownish yellow, slightly turned inwards and gradually

narrowed to the bluntly pointed apex. A pore, probably sensory, is situated near the base of the segment, while a second, bearing a short spine is present near the middle. The apex, like that of the maxillary palp, has an exceedingly minute tuft of short spines, which just break the surface of the cuticle and are visible only under the microscope.

In the basal segment there is a whorl of four moderately long setae near the apex, two of these being ventral, the others dorsal. Two pores are situtated near the inner margin of the segment, one behind the other, a third in the median line near the base, and a group of two or three pores near the base of the outer margin.

The pores found on the palps appear to be more open than those on the body of the labium.

Spiracles of Larva.

The two thoracic spiracles are situated in the pleurae of the mesothorax. They are larger than those of the abdominal segments but are otherwise similar in structure. They lie almost, if not quite, parallel to the longitudinal axis of the larva.

The abdominal spiracles, which are present in each of the abdominal segments 1 to 8, are placed in the anterior third of the dorsal scutum on either side, just above the longitudinal groove separating the tergite from the epipleura. In their case the long axis of the spiracle does not lie parallel to that of the long axis of the larva but is raised anteriorly so as to form an angle of some 45 degrees.

The shape of the complete spiracle is elongate oval (Plate IV, fig. *d*) and it is broader in proportion to its length in *A. obscurus* than in *A. sputator*. Each spiracle is provided with two slit-like orifices, between which there is a wide septum, widest at the surface (Figs. 3 *a*, 3 *b*). The septum divides the spiracle completely into two chambers and is continued anteriorly beneath the cuticle. In this portion of the spiracle, which I have named the antechamber, there are a number of pale coloured processes or trabeculae, sometimes branched, which extend far across the lumen of each antechamber. Internally another chamber called the atrium (see Böving(3)) comes into apposition with the antechambers and receives the air from them (Fig. 3 *b*). It is not, however, so wide as the width of the two antechambers and the latter are continued a little beyond the opening of the atrium, leaving a slight *cul-de-sac* in each case. From the antechambers the atrium extends inwards (*i.e.* away from the cuticle) for a distance of about double its width to meet the trachea which carries the air thence to the main longitudinal trachea

on either side of the body of the larva. At the point of contact of the atrium and the trachea there is a slight thickening around the latter.

There are no taenidia lining the atrium, but its epithelium is composed of irregular pentagonal cells, which are plainly visible in a stained preparation, even of an exuvia. Miall and Denny (10) have described a very similar chamber with polygonal epithelial cells as existing just within the stigmatic orifices of cockroaches and Böving (3) has described it in the larvae of *Donacia*, *Hister* and an Elaterid from Java.

The margins of the two stigmatic orifices are thickened into a peritreme of stout brown chitin, the surface of which, as well as the sides and

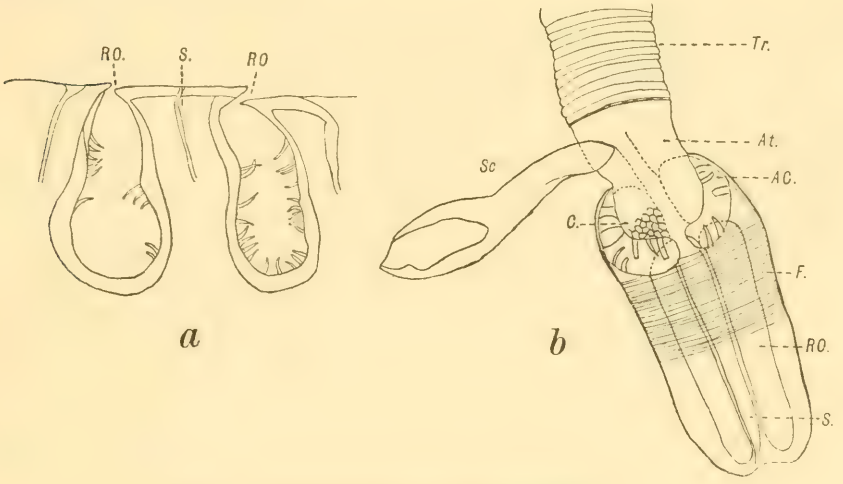


Fig. 3 *a*. Transverse section of larval spiracle.
b. Larval spiracle as seen from beneath.

Sc. = Scar; *S.* = Septum; *RO.* = Respiratory orifice; *F.* = Floor of spiracle; *AC.* = Antechamber; *At.* = atrium; *Tr.* = Trachea; *C.* = polygonal cells of atrium (a few only shown).

floor of the orifice itself, is transversely corrugated. A fine, somewhat irregular, suture separates the peritreme of each orifice on the surface of the septum. At the anterior and posterior ends of the orifice, the peritreme is narrower, especially at the posterior end. In young larvae of the first and second instars the peritreme is invisible at the anterior end, though present as a narrow ridge of chitin at the posterior end.

The corrugations already mentioned which project as small teeth on either side of the stigmatic orifices, vary in number according to the instar. In the final instar they number some 47 in the thoracic and 40–43 in the abdominal spiracles. In the young larva of the first instar they

number only about seven to eight in the thoracic and five to six in the abdominal spiracles.

Towards the anterior end of the atrium there is attached an apparent strut, connecting the atrium with the outer cuticle near the anterior end of the spiracle but a little more dorsal and running at right angles to the axis of the spiracle. This "strut" is moulted with the exuvia. It is not present in the first larval instar, although present in the second and subsequent ones. Evidently it is the scar left by the withdrawal of the trachea at the last preceding ecdysis, as was pointed out by Schiödte (12, at p. 493). At the point where the trachea has been withdrawn through the cuticle there is an oval scar of a brownish colour at the margin and sometimes bearing a septum of similar colour through its long axis. The central area of this scar is covered with yellow chitin, similar to the general colour of the cuticle and was evidently deposited subsequently to the withdrawal of the trachea.

At ecdysis the position of the spiracle is moved back a little and the lining of the atrium, with a considerable length of tracheal lining from beyond it, comes away with the exuvia, leaving the scar in the body of the newly moulted larva anterior to the new spiracle. This moving back of the spiracle at each ecdysis does not however imply any change in its position in each instar relative to the other parts of the body, as measurements have shown. The extent of the change of position therefore merely corresponds to the increase in growth of the larva.

The necessity for some provision to avoid the difficulty of extracting whole a tracheal tube through a biforous stigmatic opening is manifest. This phenomenon of the growth of a completely new spiracle, alongside the old, appears to resolve the difficulty.

De Meijere (9) found somewhat similar remnants of spiracles in the case of amphipneustic dipterous larvae, although in the cases dealt with by him the cause was apparently the covering of the stigmatic orifice by a membrane, usually complicated, which could scarcely be renewed after ecdysis had taken place.

Böving (3) deals with the anatomy and functions of biforous spiracles in coleopterous larvae and speaks of a "spiracular slit" near the spiracle in *Hister*, which he figures, as well as sections of the spiracles of a larval Elaterid from Java. In neither of these cases do his sections show the "spiracular slit" completely closed, but probably in both cases it represents the scar of the trachea just described.

Scott (13) found biforous spiracles of very similar structure in the larva of *Epuraea depressa*, but he does not describe any scar.

Not infrequently larvae are found having one or more "blind"

spiracles with no opening to the exterior but merely a brown spot of hardened chitin indicating the position of the atrophied spiracle. Probably a functional spiracle would reappear after the next ecdysis, but no investigations into this subject have been made.

Pupa.

The pupa has been described by Curtis(4), Beling(2), Ford(5) and others. A few notes on certain points which require amplification are therefore all that it seems necessary to add.

At the base of the pronotum on either side of the median suture a small papilla is placed. These do not bear spines. Nine abdominal segments are visible, the external genitalia being seen in their pupal sheaths in apposition to the ventral surface of the ninth.

In the male, close to the margin of the 8th sternite, there is a rounded and slightly notched flap, which probably represents the 8th sternite of the adult. Posterior to this flap and arising from beneath it are three rounded lobes, the median one not extending so far posteriorly as those on either side of it. Probably these represent the median and lateral lobes of the male external genital organs (Fig. 4 *a*).

In the female (which was the sex described by Ford) the 8th sternite is slightly produced into a blunt point behind and bears two small punctures side by side near the apex. From beneath the 8th sternite the pupal sheath of the ovipositor arises. This is elongate, subparallel and has a deep suture in the median line. At each of the posterior angles there is a small pointed papilla, which is the sheath of the tactile process at the apex of the ovipositor.

The apex of the ovipositor is almost co-terminous with that of the 9th tergite (Fig. 4 *b*). The small spurs observed by Ford (p. 108) at the base of each posterior spine, though frequently present, are not constant and are sometimes present on one side only. Ancillary unpaired spines may also occur on the cerci or even on the median posterior portion of the segment.

In both sexes the sternite of the 7th abdominal segment is produced posteriorly, so as partly to cover the next segment. In the adult the 7th is the last visible segment and the sternite is similarly produced to a blunt point posteriorly. The preceding sternites (2–6) of the pupa have their anterior and posterior margins subparallel as in sternites 3–6 of the beetle. The 1st sternite is very narrow and is represented by a mere fold of the integument. Both tergites and sternites of the 2nd to the 8th abdominal segments are produced laterally a little at the posterior margin, but not so much as to conceal the spiracles.

The apex of the elytral sheath is slightly hooked but less so than in some allied species (*e.g. Athous haemorrhoidalis*). The number of spiracles is the same in the pupa as in the larva. The single thoracic pair is situated ventrally in the membrane between the pro- and meso-thoracic segments, anterior to the intermediate coxae. The abdominal spiracles are situated laterally in the pleurae between the folds of the tergite and sternite, but are not concealed as in *A. sputator*. Those of the first segment are almost in the middle of each pleura, the remainder, on segments 2 to 8, are near the anterior margin of the pleura.

The spiracles are uni-forous, almost circular and bear on their

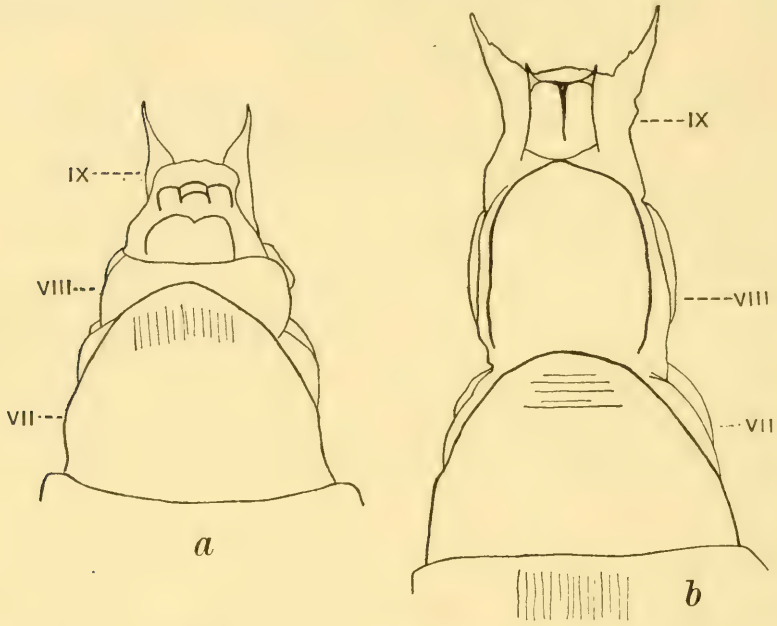
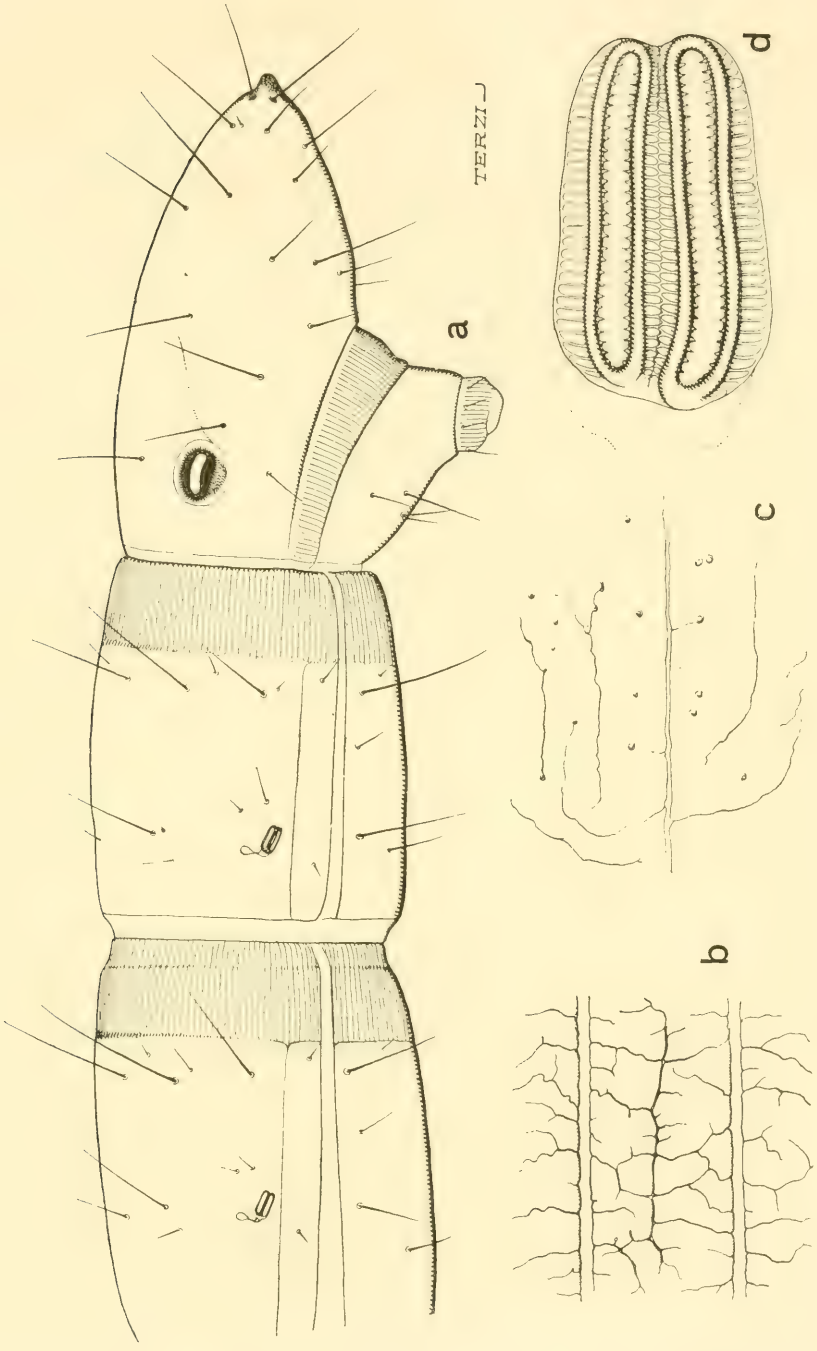


Fig. 4. Terminal segments (*a*) of ♂ pupa, (*b*) of ♀ pupa, from ventral surface.

posterior margin somewhat dorsally a small round scar with a fine linear cicatrix in the middle. There is no appearance of any atrium and the taenidia extend to the aperture.

It is interesting to compare the segments and spiracles of the pupa with those of the adult. In the latter there is one pair of spiracles more than in the pupa or larva. There are the same number (eight) in the abdomen, but two pairs in the thorax, situated (1) ventrally, in the membrane between the pro- and meso-thoracic sternites, on either side of the prosternal process and (2) dorsally, in the pleurae of the meta-thorax, beneath the insertion of the wings.


TERZI
a
b
c
d

Again, in the abdomen of the adult beetle there are normally visible seven tergites dorsally and but five sternites ventrally, the first two sternites being suppressed beneath the third. Within the 7th segment the 8th is concealed for the greater part of its length. In the female the ovipositor arises from this segment and in the male there are evidences of two rudimentary segments beyond it, as Verhoeff found(15), making ten in all. Stein(14) made out nine abdominal segments in the female, considering the ovipositor to be formed of the 9th tergite and 8th and 9th sternites. The eighth, however, is the last segment bearing a pair of spiracles in both sexes, so that the remaining two segments, if true segments, must constitute parts of the ovipositor. The "Vaginal palpe," or tactile process on either side of the aperture of the oviduct, is the terminal one according to Stein.

Therefore though there is considerable similarity between the form of the first seven abdominal segments in the pupa and the adult, the segments posterior to the 7th are entirely different, continuing to taper gently to the apex of the 9th in the pupa, while in the adult they are much narrowed and normally contracted within the body of the beetle.

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EXPLANATION OF PLATE IV

Agriotes obscurus, L. larva in late stage.

a. Last three segments.
b. Cuticle of 9th abdominal segment, between the sensory pits (much enlarged).
c. Cuticle of 8th abdominal segment along the medio-dorsal suture (much enlarged).
d. Spiracle.

(*Received July 14th*, 1921.)

NOTE ON THE CHEMOTROPISM OF THE HOUSE FLY

By W. R. G. ATKINS, Sc.D.

(*Sometime Experimental Officer, Royal Flying Corps.*)

The following notes have been called forth by the perusal of two recent papers in this *Journal*, viz. those by Imms and Husain (1920) and by Speyer (1920). The observations were made mainly at Aboukir, on the north coast of Egypt between 1916 and 1919. As may be imagined, the aim in view was the destruction of the flies, and the haphazard experiments were carried out in addition to much other work and with no thought of publication. They, however, appear to confirm the observations of the above-mentioned investigators by trials on a large scale.

It was found that formalin, when dilute, was at times very effective if exposed in a shallow dish near a window. The presence of a little bread or something to give the flies an alighting ground increased its usefulness. Flies apparently drink in the morning[1], as early as possible, and for this reason the formalin should be available at that time. When allowed to stand in the light, formaldehyde changes into one of its solid polymers, as may be noticed when the solution evaporates. The latter, however, appears to be poisonous to flies, though no direct observations were made on this point, a little formalin always being present. As pointed out by Morrill (1914) the behaviour of formalin in attracting flies is very variable. If too strong they appear to be driven away and in the presence of miscellaneous foodstuffs they will usually leave a formalin solution untouched.

As noted by other observers, beer is a very attractive substance to flies. It was found that dome-shaped glass vessels with a rim turned up inwards made excellent traps when the annular trough was filled with beer. The domes were supported on three legs and placed for preference in the sun or a well-lighted position. The number of flies caught in such traps within two hours of their first being tried at Aboukir was quite amazing. These traps were not, however, available in any quantity, nor were the Japanese clockwork traps, so attempts were made to render weak formalin more attractive by adding cane sugar, also

[1] The water supply of the numerous beetles inhabiting the patches of baked clay in the coastal deserts was a puzzle to the writer till it was noticed that they drank drops of dew from the angles of dried-up thorny plants.

ethyl alcohol. Beer, however, was much better as a lure, and it was also noticed that the addition of a little Egyptian made alcohol was very effective. Dishes containing about 1 per cent. formalin and a little of this crude alcohol compassed the death of many thousands of flies in our camp. Doubtless the amyl alcohol, of which, together with the other fusel oil constituents there appeared to be a noticeable amount in this product, attracted the flies, and some traces of esters of these alcohols may also have been present.

Speyer has drawn attention to the attraction that iso-amyl acetate and other esters of these alcohols have for flies. There is no doubt that it is very powerful, for our "doping" sheds were usually infested with flies even when neighbouring sheds or hangars were comparatively free from them. Now aeroplane "dope" consists of acetyl cellulose dissolved in a mixture containing acetone, methyl-acetate, methyl alcohol, ethyl alcohol and benzene as solvents or diluents and benzyl alcohol as a residual solvent to keep the film pliable. Though neither benzene, acetone, methyl nor ethyl alcohol attract flies in any noticeable manner, the methyl acetate may have a decided positive effect, and it is just possible that benzyl alcohol may also, but this is rather unlikely, as shown by Speyer.

Far greater however than the attraction of dope was that exerted by the nitro-cellulose varnish, in which the main solvent was iso-amyl acetate at first, and later on *n*-butyl acetate, the *n*-butyl alcohol having become available as a result of the preparation of acetone by a bacterial process. The thousands of flies attracted from a neighbouring village by the strong odour of butyl acetate afforded an example of chemotropism on a very large scale.

The considerable supplies of *n*-butyl alcohol produced in the preparation of acetone by the fermentation method have resulted in this alcohol becoming available in surplus, so its utilization in quantity as the acetate in a campaign against flies ought to be possible. The ester is volatile, and boils at 125° C., so it would have to be replenished at intervals. Iso-amyl acetate is slightly less volatile and boils at 139° C.

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(*Received April 4th*, 1921.)

REVIEW

Annales du Service des Épiphyties. Edited by Marchal, P. and Foex, E., Tome VI, pp. 1–365. Mémoires et Rapports présentés au Comité des Épiphyties en 1918. Ministère de l'agriculture, Paris, 1919.

The above volume contains much useful information regarding investigations carried out in France on insect pests and fungoid diseases of plants.

A brief report on Phytopathology in France for the year 1918 introduces the volume (pp. 1–33), and reports on the activities at the various entomological and vegetable pathology laboratories in France occupy the concluding pages.

Of the papers on entomological investigations, Professor F. Picard, Professor of Zoology at the National School of Agriculture at Montpellier, gives an exhaustive study of the entomological fauna of the fig-tree, pp. 34–174. The paper is divided into two sections, I, Special part; II, General part.

The first part deals with the more important insects which attack the wood, the leaves and the fruit respectively. Description of the insects, ample biological notes, their enemies and parasites are given.

Only one species of Lepidoptera (*Simaethis nemorana* Hubn.) frequents the fig tree in France, a fact which is discussed in Part II of the paper.

Part II consists of a general discussion on the relation of plants to insect population. The views expressed are largely formed from extensive observations on certain insects associated with cultivated plants, especially with regard to the fig tree and exotic plants in general. The question of the introduction of pests into new countries and the reasons for their subsequent wide distribution, are discussed.

Certain species of plants are populated by many species of insects, while others may only support one or two species. Chemotropic influence of the odours of plants, probably is the most important factor concerned. The significance of physical factors such as light, temperature, humidity etc. are discussed in relation to the insect population of plants.

The factors which are at work in keeping down insect pests are numerous and complex in their inter-relationship. Parasites, epidemic diseases, birds, and climatic conditions all play their part.

Cultivation carries with it a great quantitative and qualitative modification of the fauna of plants. A chapter is given on the relations between the parasites of insects and their hosts.

Professor P. Marchal gives an account of arsenical sprays and the treatment of fruit trees in America and France, pp. 242–280.

A section is devoted to the relation of arsenical sprays to the consumer of fruit. The results of experiments quoted, show that owing to rain and atmospheric conditions generally, the danger is negligible. Obvious precaution is necessary after spraying with regard to cattle and herbs which grow in orchards. Observations and recommendations based on experiments carried out in 1917 are given and an extensive bibliography is appended.

Among the mycological papers Professor E. Foex, Director of the Vegetable Pathology Station in Paris, gives detailed observations of *Leptosphaeria herpotrichoides*, the causative organism of a disease of wheat, pp. 200–213. The practical application of the results of the investigations are summarized.

Professor L. Ravaz, Professor at the National School of Agriculture, Montpellier, in an account of experimental research on the treatment of vine mildew, pp. 281–288, gives much useful information of practical value, especially with regard to the effect of spraying formulae on the plant and on the fungus.

G. Arnaud has given notes and descriptions of some new or little known diseases in France. The author notes the development of *Rhizoctonia solani* on potatoes, due to the favourable dry season, while on the other hand *Phytopthora* has been greatly restricted.

An interesting article on the destructive action of fumes from the Chedde factory in Savoy is written by Professor L. Mangin, pp. 187–199. The author shows that *Hydrochloric* acid, which are the harmful fumes, exerts the greatest effect during foggy weather, and least during dry periods.

Several other mycological and entomological articles complete this most interesting and valuable volume.

J. D.

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